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FREDERIC VINCENT THEOBALD, M.A. (Cantab.), F.E.S., Hon. F.R.H.S., was born at Kingston-on-Thames, in 1868, and was educated at St. John's College, Cambridge.

He is a pioneer in zoological work, especially in relation to Medicine, Agriculture and Horticulture, and rendered a service of inestimable value to the study of Tropical Medicine when he prepared, for the Colonial Office and the Royal Society, his ' Monograph on the Culicidae of the World,' the first attempt ever made to classify all known species of mosquitos.

In addition to this, he has made many other contributions to the study of economic zoology, including ' The Insect and other Allied Pests of Fruit ' ; ' A Text-book of Agricultural Zoology ' ; ' The Insect Life ' ; and numerous Reports on Economic Entomology, issued by the South-eastern Agricultural College, Wye, during a number of years. For many years he has also devoted special attention to the Aphididae, and has increased our knowledge of this obscure group in many ways. He was also associated with Stephens and Fantham in the authorship of ' The Animal Parasites of Man.'

He was one of the first members of the Staff of the South-eastern Agricultural College, which he joined in 1894, and from 1900 till 1903 he was in charge of the Economic Zoology section of the British Museum. He received the Imperial Ottoman Order of the Osmanieh from the Sudan Government, in recognition of his scientific work; the Grand Medal of Isidore Jeoffrey St.-Hilaire of the Société Nationale d'Acclimatation de France; and, in 1913, the Mary Kingsley Medal of the Liverpool School of Tropical Medicine. He is a Member of the Honorary Committee of Management of the Imperial Bureau of Entomology (Colonial Office) and represented the Australian Commonwealth at International Scientific Congresses.



Geo. V. Theobald

'THE DEVELOPMENT OF *ONCHOCERCA VOLVULUS* IN *SIMULIUM DAMNOSUM*

BY

D. B. BLACKLOCK

(From the Sir Alfred Lewis Jones Research Laboratory, Freetown,
Sierra Leone)

(Received for publication, 4 November, 1925)

PLATES I-IV

INTRODUCTION

The mode of transmission of various species of the genus *Onchocerca* has been a subject for speculation and experiment for many years. In the case of *O. gibsoni* and its allies in cattle particularly, much work has been done in order to discover the means of transmission, on account of the important economic results which necessarily follow the infection of meat with worm nodules. In the case of *O. caecutiens* in Central America great attention has been paid to the severe eye symptoms which the worms in the nodules have been credited with producing. The insect-borne theory of Onchocerciasis, the water-borne theory, and the theories of soil and direct contamination have each had their supporters. On the whole the tendency is to believe that an insect-borne theory best suits the facts. The following are some of the arthropods which have been suggested or experimented with for various species of *Onchocerca*. Doubtless there are others, but at present it is not possible to give a complete list.

O. VOLVULUS. *Glossina palpalis*, *Glossina longipalpis*, *Pediculus capitis*, *Pediculus humanus*, *Phthirus pubis*. *O. CAECUTIENS*: *Simulium samboni*, *Simulium dinelli* suspected by Robles. *O. GIBSONI*: *Haematopinus vituli*, *Trichodectes scalaris*, *Haematopinus eurysternus*, *Culiselsa vigilax*, *Culicoides subnitidus*, *Tabanus gregarius*, *Tabanus nigrotarsis*, *Stomoxys calcitrans*, *Lyperosia exigua*,

Haematopinus tuberculatus, *Musca domestica*, *Pycnosoma dux*, *T. cinerescens*, *S. elongatus*, *Silvius sordidus*, *Silvius* sp., *Tabanus lineatus*, and *T. near circumdatus*.

The investigation of which an account is given here was commenced in 1923-24 and continued in 1925, the whole of the work being done in the Konno district of the Sierra Leone Protectorate. In the first journey in this district the presence of cutaneous larvae corresponding in morphology to those of *O. volvulus* was observed while examining scabies (craw-craw) papules. The technique used was identical with that used by O'Neill in 1875, with the exceptions that a safety razor blade did service for a scalpel and a single section was made. O'Neill was examining the papules of Craw-craw and found skin microfilaria present in numbers; he gave a brief description of them and considered them to be the cause of this condition. The description corresponds so far as it goes with that of *O. volvulus* larvae. He describes his technique as follows:—'I find the readiest way to procure the filaria is to take between the finger and thumb a fold of the skin, so that the papule will be the highest point, then with a very sharp scalpel to slice off the epidermis, which may be discarded; now take another slice, which will remove the base of the papule and the cutis vera.'

In the Konno district not only were the larvae present in diseased and healthy skin in many persons, but the somewhat widespread prevalence of subcutaneous nodules was noted. The fact that the larvae were not found in the blood in persons whose skin was often heavily infected, made it probable that if any blood-sucking arthropod was the vector it would be one which in the process of penetrating to reach blood would rasp or tear the skin, with the result that the larvae would be dislodged into the wound and would then be taken up with the blood.

The Congo floor maggot *Auchmeromyia luteola* appeared well-adapted to dislodge larvae in the skin on account of the damage which it does before reaching blood. It is prevalent in the Konno houses, living in the mud floor; a number of Congo floor maggots were dissected without finding larvae in any of them.

At several villages in December, 1923, and January, 1924, it was noted that *Simulium damnosum* was biting in great numbers beside the small streams. They were a constant source of annoyance to

the boys who were then engaged in collecting snails for examination for cercariae and there was a continual slapping of legs to kill the fly, chiefly about the calves and ankles. In several cases blood could be observed trickling down the skin, especially when wet. The fact was noted, however, that after the fly had settled on the skin there was a considerable lapse of time before the person attacked felt any irritation and before the insect began to distend with blood. It appeared probable that this delay was due to the insects having some difficulty in piercing the skin, and this in turn suggested damage to the skin and the possible dislodgment of skin larvae if present. A hundred specimens of this species were dissected in order to see if any larvae could be discovered in the gut, but without any success. The investigation could not be continued at this time owing to pressure of other work.

On returning in 1925 it was determined to make a more extensive investigation in the Konno country in order to discover whether *Simulium damnosum* is capable of transmitting *O. volvulus*. The results of the preliminary blood and skin nodule investigations on the population are given here and also the results of the experiments so far carried out with a view to ascertaining the capacity of *Simulium* to act as vector.

A. OBSERVATIONS ON HUMAN ONCHOCERCIASIS

I. *Examination of human skin for the presence of O. VOLVULUS larvae.*

In proceeding north into the Protectorate, the skin of the villagers was examined in as many cases as possible for the presence of larvae and the occurrence of subcutaneous nodules. At a village called Tumbudu in the Konno district the conditions as regards skin infection with larvae, the occurrence of nodules in the subcutaneous tissue, and the prevalence of *Simulium* seemed favourable for the investigation and work was at once commenced here.

Agamofilaria streptocerca. This microfilaria described by Macfie and Corson in the year 1922 in natives of the Gold Coast was not discovered in the skin of the villagers at Tumbudu, where the only skin microfilaria found was that of *O. volvulus*.

Number of persons examined.

The number of natives whose skin was examined in one or more sites on the body was 123 ; of these persons fifty-two, that is over 42 per cent., had larvae of *O. volvulus* in their skin.

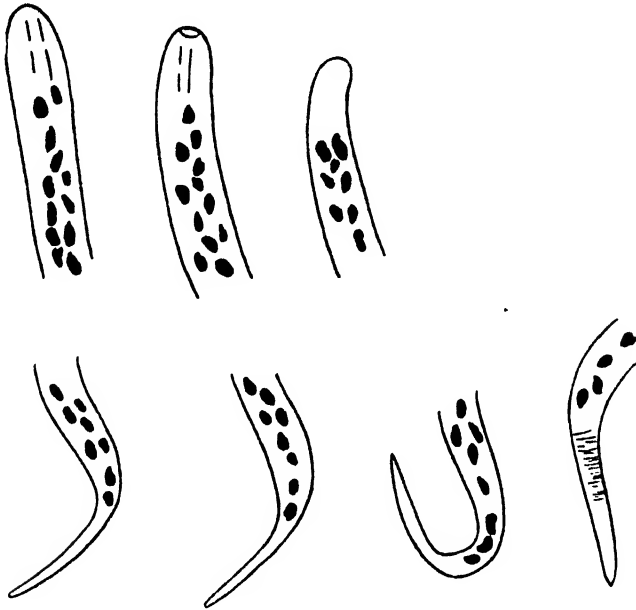


FIG. 1. *O. volvulus* forms in human skin.

Owing to the reluctance of females here to being examined by section of the skin, it was necessary to confine attention to males practically without exception. It is impossible, therefore, to make any comparison between the prevalence of *O. volvulus* larvae in the skin of the male and female sexes.

Number of sections of skin examined.

The total number of sections of skin examined from the 123 cases was 441 ; of these sections 102, that is over 23 per cent., were positive.

Distribution of O. VOLVULUS larvae over the body.

In a number of cases a more systematic examination of the body was possible ; pieces of skin were examined from four regions of the body, viz. : the scapular region, the loin, the thigh and the

ankle. Of ninety-three persons in whom this method was adopted forty-two, that is over 45 per cent., gave a positive result as regards the larvae of *O. volvulus* in one or more of the four sites. The findings in the different regions are tabulated below.

TABLE I.

Showing the distribution of *O. volvulus* larvae in 93 persons, each examined in four regions of the body.

| Total persons examined | Total positive | Regions where <i>O. volvulus</i> larvae were present in the skin | | | |
|------------------------|----------------|--|------|-------|-------|
| | | Scapula | Loin | Thigh | Ankle |
| 93 | 42 | 19 | 32 | 24 | 5 |

It is seen from the table that the waist region is that one of the four regions examined in which the larvae were most commonly found.

II. *Condition of skin considered in relation to presence in, or absence from, it of O. VOLVULUS larvae.*

The observation made in the previous tour that *O. volvulus* larvae were frequently present in portions of skin which looked perfectly healthy was fully confirmed by the present series of examinations.

Special attention was paid to the condition of the skin in sixty selected cases referred to later. It may be mentioned here that the majority of these cases were members of the more important families of the village; that they were therefore better fed, better clothed, better washed and better housed than the average population. The condition of the skin in these cases was strikingly superior to that of the casual cases, who were often of a poorer labouring class. It was thus possible to find among these sixty cases seventeen whose skin was classed as very good, that is to say, that a complete examination of the body did not reveal any lesions of scabies, ulcers, or other obvious disease of the skin.

Of these seventeen cases having very good skins no less than eight had larvae of *O. volvulus* in sections made from various portions of the body. The youngest person having numerous larvae in a very good skin was twenty-four and the oldest fifty-one.

It appears evident, therefore, that the presence of *O. volvulus* larvae in the skin does not by any means always connote obvious

disease of the skin, even in people of advanced years ; whether this skin infection, if uncomplicated, does eventually produce obvious disease is a question which it appears impossible to answer at present.

III. *The existence of subcutaneous nodules considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

Sixty-eight cases were examined as to the presence of larvae in one or more skin sections, and subcutaneous nodules other than glands. The results are given in Table II.

TABLE II.

Showing the percentage of subcutaneous nodules other than glands in those having, and in those not having, *O. volvulus* in the skin.

| Total examined | <i>O. volvulus</i> in skin | <i>O. volvulus</i> not in skin | Nodules present | Nodules absent | Percentage with nodules |
|----------------|----------------------------|--------------------------------|-----------------|----------------|-------------------------|
| 68 | 30 | — | 12 | 18 | 40 |
| | — | 38 | 6 | 32 | 16 |

It is seen that only 40 per cent. of those having *O. volvulus* larvae in the skin had also subcutaneous nodules other than glands, while of those not having *O. volvulus* larvae in the skin 16 per cent. had subcutaneous nodules other than glands.

IV. *Condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

The same group of sixty-eight persons was examined for enlarged glands ; in Table III are set out the figures obtained.

TABLE III.

Showing the percentage of enlarged glands in those having, and in those not having, *O. volvulus* larvae in the skin.

| Total examined | <i>O. volvulus</i> larvae in skin | <i>O. volvulus</i> larvae not in skin | Enlarged glands present | Enlarged glands absent | Percentage with glands |
|----------------|-----------------------------------|---------------------------------------|-------------------------|------------------------|------------------------|
| 68 | 30 | — | 8 | 22 | 27 |
| | — | 38 | 12 | 26 | 32 |

It is seen that only 27 per cent. of those having *O. volvulus* larvae in the skin had also enlarged glands, while of those not having *O. volvulus* larvae in the skin 32 per cent. had enlarged glands.

V. *The existence of subcutaneous nodules and the condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

If we consider these sixty-eight cases from the point of view of the occurrence of either subcutaneous nodules or enlarged glands or both in cases with and without *O. volvulus* larvae in the skin, the figures given in Table IV are obtained.

TABLE IV.

Showing the percentage of persons with subcutaneous nodules or enlarged glands, or both, in those having and in those not having *O. volvulus* larvae in the skin.

| Total examined | <i>O. volvulus</i> larvae in skin | <i>O. volvulus</i> larvae not in skin | Both glands and nodules | Either glands or nodules | Neither glands nor nodules | Percentage with either nodules or glands or both |
|----------------|-----------------------------------|---------------------------------------|-------------------------|--------------------------|----------------------------|--|
| 68 | 30 | — | 2 | 15 | 13 | 57 |
| | — | 38 | 2 | 14 | 22 | 42 |

It will be observed that nodules or glands or both are present in 15 per cent. of those who have larvae in the skin, in excess of those who have not larvae in the skin. The margin is probably not sufficient to permit of arriving at any general conclusion as to the relationship between glands and nodules on the one hand and skin infection with *O. volvulus* larvae on the other.

VI. *The condition of the eyes considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

In view of the fact that profound ocular changes are stated to be due to the action of *O. caecutiens* in Central America, attention was paid to the eyes of certain cases which came for examination.

In a series of twenty-three unselected cases the vision was tested and the eyes were examined externally for evidence of disease; in the same cases the skin was examined for the presence of *O. volvulus*

larvae. The tests of vision utilised for these illiterate natives were two, for near vision the threading of a sewing needle, and for distant vision the counting of a number of pieces of stick held at different distances. The only abnormalities found were :—slight exophthalmos without visual defect, two cases ; pterygium coupled with defect of vision approximately R $\frac{3}{6}$ L $\frac{1}{6}$, one case ; chronic blepharitis oedema of sclerotic and pterygium coupled with defect of vision R $\frac{3}{6}$ L $\frac{2}{6}$, one case ; history of discharge from eyes at intervals for one year, one case.

The results of the examination of the eyes and of the skin are set out in Table V.

TABLE V.

Showing the results of eye examination and of skin examination for *O. volvulus* larvae in 23 unselected cases.

| Total examined | Skin examination | | Eye examination | |
|----------------|------------------|---------------|-----------------|---------------|
| | Larvae present | Larvae absent | Eyes normal | Eyes abnormal |
| 23 | 11 | — | 7 | 4 |
| | — | 12 | 11 | 1 |

At first sight it might appear that there were more abnormal eyes among persons having *O. volvulus* in the skin than in those not having these larvae in the skin. It appears possible, however, that this apparent difference may to some extent be explicable on the ground of age. The persons who had larvae in their skin in this series happened to be much older than those who had no larvae in the skin. Thus the average age of the seven having normal eyes and larvae in the skin was forty years and that of the four having abnormal eyes and larvae in the skin was forty-seven years ; whereas the average age of the eleven having normal eyes and no larvae in the skin was twenty-six and the age of the case having abnormal eyes and no larvae in the skin was twenty-five.

A series of thirty-eight cases selected in age periods gave a different result as seen in Table VI.

TABLE VI.

Showing the result of eye examination and of skin examination for *O. volvulus* larvae in 38 selected cases.

| Age | Total examined | Skin examination | | Eye examination | |
|-----------|----------------|------------------|---------------|-----------------|---------------|
| | | Larvae present | Larvae absent | Eyes normal | Eyes abnormal |
| | 38 | 18 | — | 18 | 0 |
| | — | — | 20 | 19 | 1 |
| 0 to 10 | 8 | 0 | 8 | 8 | 0 |
| 11 to 20 | 3 | 1 | 2 | 1 | 0 |
| | — | — | — | 2 | 0 |
| 21 to 30 | 9 | 6 | 3 | 6 | 0 |
| | — | — | — | 3 | 0 |
| 31 to 40 | 6 | 3 | 3 | 3 | 0 |
| | — | — | — | 3 | 0 |
| 41 to 50 | 5 | 4 | 1 | 4 | 0 |
| | — | — | — | 1 | 0 |
| 51 over | 7 | 4 | 3 | 4 | 0 |
| | — | — | — | 2 | 1 |
| TOTAL ... | 38 | 18 | 20 | 37 | 1 |

In this series only one case had abnormal eyes, a condition of pterygium with history of discharge from the eyes periodically: vision good.

No evidence of such conditions as irido-cyclitis, keratitis punctata, or panophthalmitis was obtained in either of these series. On the contrary the general appearance of the eyes and the visual acuity even in the older cases was excellent. In this locality, therefore, it was not possible to obtain evidence which would justify any etiological association between disease of the eyes or defects of vision and the infection with *O. volvulus* as determined by skin and nodule examination.

VII. *Character of the subcutaneous nodules.*

These varied from a smooth, soft, just palpable nodule to a large, irregularly nodular, hard mass of the size of a large walnut. The first type was usually found in the region of the great trochanters, on the ribs (Case 50), and on the scalp; the second type was generally closely associated with joints, more especially the elbow and knee joints; neither type was usually adherent to the skin, but in some cases, especially over the great trochanter region, the skin over the nodule was thickened, partly adherent, and polished on the surface from pressure.

Pain was frequently complained of, and some persons who had nodules on both trochanter regions had resorted to the use of a circular ring-pad resembling a magnified bunion pad to enable them to sleep on the side.

Cases were examined in which larvae were found in the skin without palpable nodules being present; cases were found where *O. volvulus* larvae were present in the skin while the nodules which were present gave negative results on puncture; cases were found where *O. volvulus* larvae were present in the skin and also in nodules; there were no cases in which, although no larvae were found in the skin, puncture of a nodule gave a positive result.

Juxta-articular nodules.

If the region had not been one in which *O. volvulus* was present, several of the cases seen would have been classed as juxta-articular nodules.

The examination of puncture fluid by no means always cleared up the diagnosis. In this connection special reference may be made to two cases.

CASE 59. This was a man of fifty-five years of age, whose skin was apparently normal except over the knees and elbows, where it was much wrinkled; the eyes were free from disease and the visual acuity was good; the blood was negative. On both sides the inguinal glands were enlarged. He had the following nodules:—

| | | | | | |
|------------------------|-----|----|------------|------------------------------------|--------|
| Near R trochanter | ... | 2 | each about | $1\frac{1}{2} \times 1\frac{1}{2}$ | inches |
| L ,, | ... | 2 | ,, | $1\frac{1}{2} \times 2$ | ,, |
| R elbow | ... | 1 | ,, | 2×2 | ,, |
| L ,, | ... | 1 | ,, | 2×1 | ,, |
| L knee (head of Tibia) | 1 | .. | 1 | $\times 1$ | .. |

These nodules were irregular in shape and had accessory small nodules at the margins. They were hard, fairly mobile under the skin, and non-adherent. They gave the impression of resulting from the fusion of several nodules.

Puncture of the left elbow nodule resulted in a flow of amber-coloured fluid oozing slowly from the needle. Puncture of one right trochanter nodule produced a few drops of serous fluid; the puncture of the remaining nodules gave small amounts of an opalescent fluid with light brown granules in it. In addition to each nodule being punctured once, two of the nodules, one on the right trochanter and one on the left trochanter, were punctured a second time in a different place. None of these nine punctures revealed the presence of either eggs or larvae. It would perhaps have been legitimate to conclude that these nodules were not connected with infection with *O. volvulus*. Such a conclusion, however, appeared less justifiable in view of the fact that the examination of skin sections revealed a particularly heavy infection with *O. volvulus* larvae in the scapular, loin, and thigh regions, although not in the ankle region; gland puncture was not permitted.

CASE 51A. Here the nodules were not so numerous but they were of a similar character and gave an even more interesting result. The nodules were:—Right femur internal condyle, two; right trochanter region, two. Puncture of the four nodules gave a negative result for three and a positive result for one, namely, one of those over the condyle of the femur. The blood was negative, but the skin of scapular, loin, thigh and ankle regions was heavily infected with larvae of *O. volvulus*.

B. OBSERVATIONS ON *SIMULIUM DAMNOSUM*

A. GENERAL.

Hour of day at which biting.

Observations were made at various hours of the day in order to ascertain at what hours the fly was prepared to feed in this locality and whether any particular hours were preferred by it. It was found that individuals were not captured biting earlier than 6 a.m. and that only the latest stragglers were biting as late as 6 p.m. In the

middle of the day the fly would not go far in order to bite, not even five yards as a rule. The flies were found biting, however, in bright sunny weather at any hour of the day, provided they had only a yard or two to go from their shelter. Even on sunny days they were thus captured at any hour of the day from 6 a.m. to 6 p.m. On dull days without rain they bit freely at any hour of the day and a slight shower did not reduce the catch materially. On wet days, however, with heavy rain, they were not found biting, and it was noted also that soon after a heavy rain there was a considerable lapse of time before they would again come out to bite. All these records were made close to the edge of water, usually flood water from the large river or near small streams.

It was frequently noted that during a period which gave a good capture there were intervals in which the fly was not biting. They thus appeared capricious at times when no alteration was noticeable in the temperature or light ; the fly was sought out in places where it was known to exist and the collectors encouraged them to bite by disturbing their resting place in the long grass and bush and submitting quietly to their attacks. It was noticeable that the fly did not come out to attack as it was observed to do in the month of December in another locality.

Length of life in captivity.

Flies captured while just commencing to feed and kept in tubes of various sizes without food, and with or without moisture supplied, whether kept in the dark or in the light, failed to survive more than three days.

Various experiments were made in order to discover some means of keeping wild flies alive in captivity, but the results were disappointing ; the containers were covered in each case with gauze of 50-55 meshes to the inch ; this, in the case of solid containers such as those of tin and wood, made the inside dark. The containers used were of glass, metal or wood, and were of various sizes and shapes. Foods of various kinds, e.g. moist raisin, fresh banana, oranges, and particles of meat were supplied ; the condition of moisture was varied by adding damp blotting paper, damp sand or free water. Efforts to reproduce the conditions of nature consisted in placing small plants with the soil in which they were growing in several of the containers.

In spite of the efforts made, the longest period of survival in a total of 268 flies was ten days, and that a solitary instance. Individual specimens lived for eight and seven days, but by far the majority were dead within four days. On the whole, it may be said that dry glass cylinders and large test tubes with raisin as food gave better results than did either wood or metal containers, but the cases of survival were few and far between.

Effects of sunlight.

Fully-fed flies exposed in test tubes immediately after the feed to the full glare of sun just before noon died in twenty to sixty seconds after a period of violent excitement. Partially-fed flies survived for one to two minutes.

Feeding in captivity.

In spite of numerous attempts made, using various expedients such as moistening the skin, smearing with plant juices, serum or blood, no fly was ever induced to bite the human skin, either white or black, in captivity. Flies were observed to sit on moist raisin and appeared to be attempting to feed, but they did not engorge; they apparently took up a little water from time to time from the muslin at the edge of the moist food.

Time taken to feed and attitude in feeding.

1. *On human beings.* The time required by the fly to feed to repletion was noted on several occasions, when flies were observed to settle and bite and fly away unmolested. It was found that the time varied from one-and-a-half to five minutes. There is a considerable interval after the fly alights and begins to operate on the skin before any distension of the abdomen is visible; in flies captured at this point and dissected, the gut frequently contained cellular débris; the majority of the time was occupied in getting down to the blood level and after this was reached distension was a rapid process. The attitude at the commencement was with the body parallel to the skin surface, the wings flat and almost overlapping; by the time the abdomen was nearly distended the position was altered so that the whole body was at an angle to the skin with the head close down to it and the tip of the abdomen well raised from the surface.

They were easily disturbed in the early stages of their feed, but after they had reached blood and commenced to engorge, they were not disturbed by placing a test tube over them and went on feeding till distended, and they were thus easily captured. The extent to which these flies engorge is remarkable and the abdomen distends visibly owing to the large accumulation of blood in the mid-gut ; in specimens dissected at periods varying from half-an-hour to

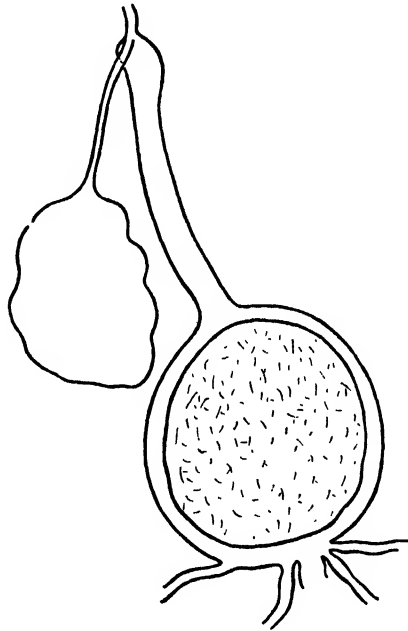


FIG. 2. Midgut of *Simulium damnosum* distended with human blood.

six hours after feeding, the blood was always found in this organ and never in the thin membranous structure which apparently corresponds to the food reservoir of tsetse-flies. The blood forms a globular mass which retains its form even after being pressed out of the cut anterior end of the gut ; the blood mass is semi-solid, sticky in consistency, and difficult to disintegrate by needles, soon after the feed ; altered blood was found in small amounts up to four days after feeding. The food reservoir was usually found to be full of a clear glairy fluid after the expression of which the thin walled sac collapsed.

2. *On animals.* Only a few experiments on animals were made, employing a sheep, two young goats, and a dog. These were taken at various times to the spots where flies were being captured biting human beings. The fly was not seen to bite the animals when these were walking about. It was possible, however, by placing the young goats on their backs to observe that two flies bit them and engorged with blood in the regions of the mammary gland and axilla. The time taken to engorge did not exceed the time found for human beings, and the degree of engorgement was the same.

Effects of rain and immersion in water during feeding.

It was found that a coarse spray of water applied to the fly while biting caused it to relinquish its hold. In the same way the direct impact of rain drops on the feeding fly dislodged it. If, however, the limb on which the fly was feeding were immersed gently in water the fly very frequently did not relinquish its hold.

1. Fly biting on leg just above ankle; whole foot and ankle gently immersed in still water so as to cover fly to a depth of two inches; fly remained and continued biting under water and swelled out; on removing foot gently the fly was still attached but almost immediately flew off.

2. In a similar experiment, the fly remained immersed for five minutes but was loose on bringing the foot to the surface.

3. A feeding fly was immersed for three minutes; on removal of the foot was still feeding.

4. A fly biting on dorsum of foot was immersed; in three minutes came up and stuck to hairs on leg just below surface of water; thirty seconds later it suddenly shot up to the surface, circled about rapidly for a few seconds on the surface and then flew off.

The fly appeared to have attached to it bubbles of air when it came up to the surface, and it made rapid gyrations on the surface, seemingly until these bubbles burst, before flying off.

Cutaneous and general reactions after bites.

When a fly settles, which it does without sound, and commences biting, nothing is noticed as a rule if the attention is otherwise occupied; in a large number of experimental observations the

person being bitten was quite unaware of it, even when the fly was engorging with blood, if the attention were otherwise engaged ; in other cases where the attention was directed entirely with the object of detecting when the fly was biting the initial stages of the bite were not noticed, and irritation was first felt when the fly commenced to draw blood.

Certain persons, few in number, failed to observe that they were being bitten even when their attention was directed to detecting bites, and the flies would engorge and go off unperceived. There is great variation in sensibility in this respect, but when flies are biting in any number even the most unobservant becomes aware of it.

The first thing noticed is a very slight scratching sensation which goes on for about a minute or more ; this is followed by a slightly painful pricking sensation and this finally by an intense irritation which comes on just as the fly is ready to move away.

If the affected part is immediately scratched the irritation quickly passes off, the lesion made by the fly having been enlarged by the scratching. In natives, even if scratching was prevented little local reaction could be seen on the skin ; they appeared to be immune ; on the skin of a white person examined, the reaction on the unscratched skin consisted of a white raised wheal about 5 to 8 mm. across with a red point in the centre ; intense irritation was felt for ten minutes, and subsequent slight irritation for about two days.

In cases where numerous flies bit close together on the skin, especially when it was damp, the lesions would occasionally ooze somewhat freely and the blood droplets would coalesce and form a drop which trickled down. Non-biting flies were seen to settle on the skin and feed on the blood in such cases.

Prevalence of fly.

Some indication of the numbers of fly actually biting when given the opportunity in their haunts at this season may be gained from the following figures ; each number given represents those caught on a single individual ; no males were captured.

| | | |
|---|-----|-----------------|
| On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m. | ... | 1 boy caught 73 |
| On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m. | ... | 1 boy caught 58 |
| On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m. | ... | 1 boy caught 55 |
| On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m. | ... | 1 boy caught 10 |

| | | | | |
|--|-----|-----|--------------|----|
| On 31.7.25, bright and sunny, 8 a.m. to 2 p.m. | ... | ... | 1 boy caught | 80 |
| On 1.8.25, dull, sunless, 7.30 a.m. to 8.30 a.m. | ... | ... | 1 boy caught | 25 |
| On 1.8.25, dull, sunless, 10 a.m. to 4 p.m. | ... | ... | 1 boy caught | 46 |
| On 24.8.25, sunny, 8 a.m. to 10 a.m. | ... | ... | 1 boy caught | 24 |
| On 25.8.25, dull afternoon | ... | ... | 1 boy caught | 30 |
| On 25.8.25, dull afternoon | ... | ... | 1 boy caught | 30 |

It is seen that here we have a fly which is so numerous and bites so freely that any individual during the course of the day may receive a very large number of bites. Even although only a small percentage of those which bite acquire or transmit a disease virus it is clear that the very large numbers would make the transmission probable, provided that subsequent biting occurs after such an interval as would permit of the development to an infective stage of the virus in the fly.

Situation on the body of bites received.

In considering the part of the body bitten it may be stated that the experiments were carried out in those places where flies were being captured, that is, in fairly open areas without much high shade and in grass about three to six feet high with a few shrubs among it. A small portion of the low grass was beaten down and the person stood or sat in the clearing. It was found, if a person stood erect with feet, legs, and thighs exposed to bites in such open situations, that about 80 per cent. of the total bites were received below the level of the knee. The fly attacked a portion of the skin which was in shade and a favourite site was just below the bulge of the calf or below the malleoli. When the person sat or squatted in the cleared area with the skin exposed, the buttocks and loins were chiefly attacked; a person standing among the grass before it was trampled down was bitten wherever the grass reached on his body. The fly did not, as a rule, leave the shade afforded by the grass and the lower parts of the trunk, and attack the face, neck and shoulders. It was observed that while sitting on a camp-stool in the centre of the small cleared area, the arms, neck and face, although exposed, were never bitten during an hour, while persons standing were bitten below the knee and those sitting on the ground were bitten on the loins, buttocks, and legs.

Habits as regards following human beings.

Native accounts describe this fly far from water and pursuing the rice harvesters on dry farms; little evidence of following was obtained however, during the period of investigation. The nearest point to the rest-house at which fly could constantly be captured was 150 yards away. There was a steady traffic of persons to and from this spot at the edge of the river, yet in the whole period only half-a-dozen flies were caught biting in the rest-house compound. Apart from the fact that persons passing through the position occupied by the fly were not followed, there is also the fact that the bush extends from the spot right up to the rest-house compound. It is seen, therefore, that little or no movement of the fly through the bush was taking place here at this time. In experimenting on this question it was found that if the fly-collector was standing or sitting in a small cleared area at the edge of the long grass, other persons could stand on the path at a distance of only five yards from this spot and hardly ever receive a bite; at two yards the bites were more numerous, more especially if the skin was moistened with water. There was thus no evidence here at this period of the year of following of human beings by the fly from those of its permanent sites, near the water, which were tested.

Types of country in which fly was found biting.

The proximity to water was the essential feature noted. In Plate II, fig. 3, is shown a river of some size on the shelving banks of which, where they were grass-covered and provided with shade from trees and shrubs, the fly could be found biting at almost any hour of the day except in actual rain or immediately after a heavy rain.

On the opposite side (Plate II, fig. 4) of this river the banks are low, and overflow was constantly occurring with each rain. Among the grass at this side the fly was constantly to be found also. The collector simply walked in from the path at any point, trod down some of the grass, beat the standing grass with his hands and, taking off his clothes, sat down. In a few minutes dozens of flies would be round him trying to select a shady spot on his skin on which to feed.

The wet rice farms also were favourite spots ; most frequently these lay on each side of small slow streams and here the rice was growing on sodden ground. There might, or might not, be overhead shade, but if not, the fly bit very low down. Some collectors found that by first wading through water and then offering their skin to the fly, they received more bites.

Habits of people in relation to bites of Simulium.

People bathing in the rivers are not frequently bitten, but those who stand on the banks of the river suffer severely. The farming operations involve both sexes ; the bush is cleared by the men, a certain amount of weeding is done by the women and both take part in cutting the rice. This process involves walking through the growing rice and cutting the tops off, the straw being trampled under foot. The clothing worn in these operations is reduced to a loin cloth, or this and a very short shirt may be worn. The places on the body which would therefore most usually get bitten are the regions below the level of the top of the growing rice, that is, from the waist downwards. Young children are employed to frighten the birds, but are perched on high pedestals erected on the farms.

In the Konno country all go to the waterside to defaecate. The waterside area is in close bush and the latrine is normally the edge of a small stream. Here the fly has an excellent and frequent occasion to bite about the waist region of both sexes. Both the habits of the fly as seen here and the habits of the people tend to produce the same effect, namely, that it is the region from the waist down that is most attacked ; possibly males are more exposed to its attacks than the females during their farming operations.

Native names.

Some of the tribal names for *Simulium* in Sierra Leone are :—

| | | |
|----------|-----|--|
| Konno | ... | Mooli or Mooyee |
| Timne | ... | Mapus |
| Mendi | ... | Mawwee |
| Lokkoh | ... | Poondée gootena, i.e. short mosquito (Poondina = mosquito). |
| Susu | ... | Koondée |
| Mandingo | ... | Mootee |

B. DISSECTION OF WILD SIMULIUM

Gut of fly.

Boys were taken to places where the fly had been observed biting and instructed to catch each fly after it had begun to feed on the skin. The flies were then dissected in order to see whether any of them were ingesting *O. volvulus* larvae, the gut alone being examined. Of a total of 780 flies which were so dissected twenty, that is, 2.6 per cent., had in their gut larvae which appeared in the fresh state to be *O. volvulus* larvae, and which on staining showed the morphological characters of this larva. The larvae were conspicuously active in the gut contents of the fly, much more so than when dissected out of pieces of skin in a drop of water on a slide. The blood was found soon after a meal to be agglomerated into an amorphous-looking, spherical mass, and the larvae could be seen actively moving chiefly between the anterior surface of this mass and the wall of the mid-gut; they were active in flies which had died six, twelve, and even eighteen hours before dissection.

It was, therefore, proved that *Simulium* is capable of taking up, in feeding, larvae having the character of the larvae of *O. volvulus*, and further, it was proved that the larvae, having been ingested, not only retained vitality but definitely increased in the activity of their movements; the latter fact pointed to the possibility of the larvae undergoing further development in the fly.

Thorax of fly.

Flies which had been captured in the same manner by the boys were examined as to the occurrence of developmental forms of larvae in the thorax. Of 1,320 flies in which the thorax was dissected developmental forms of larvae at different stages were found in fifteen, that is, over 1 per cent. This investigation showed that nematode larvae of some kind were capable of development in the thoracic tissues of *Simulium*.

Head of fly.

This was dissected in the case of 1,140 flies caught by the boys; in no case was a larval form found actually in the head, although one form was partially embedded in the head and was pulled out of the anterior thoracic region with the head.

Identity of the thoracic forms.

While, therefore, there was evidence that development of some larvae was capable of proceeding in *Simulium* there was no evidence that the developing forms were part of the life-cycle of *O. volvulus*; the only suggestive points were that, as previously stated, it had been proved that *Simulium* could readily take up larvae morphologically identical with this in feeding, and that when in the insect's gut the larvae showed increased activity and good viability.

In order to obtain confirmatory evidence bearing on this point the following examinations were carried out, the object being to determine as far as possible by a process of exclusion what the larvae were which were present and developing in the thorax of wild *Simulium* in this district.

- I. Examination of day and night cutaneous blood of selected human beings.
- II. Examination of day cutaneous blood of unselected human beings.
- III. Examination of day cutaneous blood of domestic animals.
- IV. Examination of the skin of domestic animals for microfilariæ.

Examination of day and night cutaneous blood of selected human beings.

Sixty persons, all males, were examined for microfilariæ in the cutaneous blood of the ear at noon; a thick film for each person was examined. It was intended to examine the same persons at midnight, but there was some difficulty about persuading them, so that only forty-six of them could be collected for the midnight examination.

The cases were selected so as to give ten persons in each of six age groups and the results obtained are set out in Table VII.

A single sheathed microfilaria present at noon in the film of one individual in the 41-50 age group proved to be *Microfilaria bancrofti*, the same individual at midnight being found to have numerous microfilariæ of this species present in the cutaneous blood. All of the other persons who had microfilariæ in the cutaneous blood at midnight were also infected with this parasite, no other being found.

This examination, therefore, revealed no infection with either

TABLE VII.

Showing the findings in the cutaneous blood of 60 males at noon and 46 males at midnight.

| Age period | Noon | | Midnight | |
|-----------------|-----------------|-----------------------------|-----------------|-----------------------------|
| | Number examined | Number having microfilariae | Number examined | Number having microfilariae |
| 0 to 10 | 10 | 0 | 10 | 0 |
| 11 to 20 | 10 | 0 | 3 | 0 |
| 21 to 30 | 10 | 0 | 9 | 4 |
| 31 to 40 | 10 | 0 | 7 | 2 |
| 41 to 50 | 10 | 1 | 8 | 3 |
| Over 50 | 10 | 0 | 9 | 4 |
| TOTALS | 60 | 1 | 46 | 13 |

Acanthocheilonema perstans or *Loa loa*, while it demonstrated a moderate infection of this sample of the population with *F. bancrofti*.

Examinations of additional persons at a late hour of the night were not possible owing to the reluctance of the villagers to attend. Additional cases were, however, examined at various hours of the day from soon after 6 a.m. to nearly 6 p.m., as shown in the following Table

TABLE VIII.

Examinations of day cutaneous blood of 83 unselected human beings.

| Time | Number examined | Infected with microfilariae |
|----------------------|-----------------|-----------------------------|
| 6 to 7 a.m. | 4 | 0 |
| 7 to 8 a.m. | 9 | 0 |
| 8 to 9 a.m. | 7 | 0 |
| 9 to 10 a.m. | 9 | 0 |
| 10 to 11 a.m. | 11 | 0 |
| 11 to 12 noon | 21 | 0 |
| 12 to 1 p.m. | 5 | 0 |
| 1 to 2 p.m. | 5 | 0 |
| 2 to 3 p.m. | 5 | 0 |
| 3 to 4 p.m. | 4 | 0 |
| 4 to 5 p.m. | 2 | 0 |
| 5 to 6 p.m. | 1 | 0 |
| | 83 | 0 |

It is seen that in this series also neither *A. perstans* nor *Loa loa* was found.

As a result of this examination it appeared improbable that the larva found developing in the thorax of *Simulium* was that of either *A. perstans* or *Loa loa*. It might be that of *F. bancrofti*, as the popula-

tion harboured this parasite in a fair proportion of cases. Against this, however, we have the biting habit of the fly, which was found biting here at this season only between the hours of 6 a.m. and 6 p.m. It appears possible, however, that the fly might become infected with this parasite even if its biting was entirely diurnal, because *F. bancrofti* larvae were seen once at mid-day in one case and possibly were present in the cutaneous blood of other cases in numbers which, although too small to be revealed by the examination of a single thick film, might yet be taken up by the fly in biting.

III. *Examination of day cutaneous blood of animals.*

No cattle or horses were present in the village of Tumbudu nor were any seen at any of the sites at which the boys were engaged in collecting flies.

There were a few sheep and goats and one dog in the village, and blood from the ear of the majority of these was examined in fresh film. Thirty-one sheep and nine goats were examined in this way without the discovery of any microfilariae in them. An examination of these animals was all the more necessary as it was proved that *Simulium damnosum* is capable of engorging upon goats at least.

IV. *Examination of the skin of animals for microfilariae.*

The portion of the tip of the ear of each animal from which blood was being taken was kept and teased up with needles in a drop of water on a slide and examined for microfilariae; in no case was a microfilaria found.

The general effect of the results of these examinations was to give the impression that whatever the larva was which was found developing in the thorax of wild *Simulium damnosum*, it was probably neither that of *A. perstans*, *L. loa*, or *Ag. streptocerca*, nor of a filaria of cattle, horses, goats, or sheep. The probabilities rested then between *F. bancrofti* and *O. volvulus*.

One of the direct results of this series of examinations was that it was rendered possible to make an advance by attacking the problem in a somewhat different way. There now became available certain persons of whom it was known that they did not present

larvae of *L. loa*, *A. perstans* or *F. bancrofti* in their cutaneous blood, and in whose skin only *O. volvulus* could be demonstrated. The cases which fulfilled these conditions were re-examined and two of them were selected as presenting the most intense infection of the skin with *O. volvulus*. These cases were then used to replace the previous fly-collectors; they were taken to the chosen site and each was attended by a boy whose work consisted in collecting flies off one of these two cases. The process of collecting was carefully supervised in order to avoid the possibility of the flies being captured on any other person than the two special cases.

The immediate effect of adopting this procedure was that whereas the gut infections had hitherto been 2.6 per cent. in 780 wild flies caught on the unselected boys engaged in collecting, it rose at once to 17 per cent. when the flies dissected were all collected from these two cases. At this stage the less heavily infected case was discarded and the remaining case was alone exposed to the bites of the fly, and in successive experiments this man was clothed so as to allow only certain areas of skin to be bitten.

First of all, he was clothed so as to expose to the fly only the region from the loins upwards; as a result of this the percentage of fly infection in the gut rose to 28. In the next experiment all the body was protected except the waist and hips; flies captured feeding on this area showed a gut infection percentage of 59.

Finally the case was exposed to the fly in such a way as to allow biting only on a narrow band of skin four inches wide round the hips, having the subcutaneous nodules, which were present near each trochanter, in the centre of the band. In this last experiment the percentage of gut infected flies rose to 80.

The results are shown in Table IX; the fact that the area of body exposed was more and more restricted necessarily had the result that smaller numbers of flies could be collected from the case. The blood of these cases was examined frequently, usually just before starting out to collect and immediately on return. No blood microfilariae were found other than, on two occasions, *Microfilaria volvulus* itself.

TABLE IX.

Showing increase in percentage of gut infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

| Feeding on | Number of flies | Gut infections | |
|---|-----------------|----------------|------------|
| | | Number | Percentage |
| Unselected boys | 780 | 20 | 2.6 |
| Cases 47 and 50 (whole body) | 41 | 7 | 17.0 |
| Case 50 (loins upwards) | 54 | 15 | 28.0 |
| Case 50 (waist and hips only) | 29 | 17 | 59.0 |
| Case 50 (on band of skin round hips with nodules in centre) | 20 | 16 | 80.0 |



FIG. 3. Edge of blood mass in anterior part of midgut of *S. damnosum*, with larvae of *O. volutus*.

This series of experiments not only served to confirm, amply, the observation that *Simulium* can take up the larvae of *O. volvulus* from the skin, but also showed that when selected areas of heavily-infected skin were exposed to it very large numbers of larvae were ingested. In some flies between one and two hundred *O. volvulus* larvae were counted in the gut. It seems probable that when this species of *Simulium* feeds on an area of skin affected with *O. volvulus*

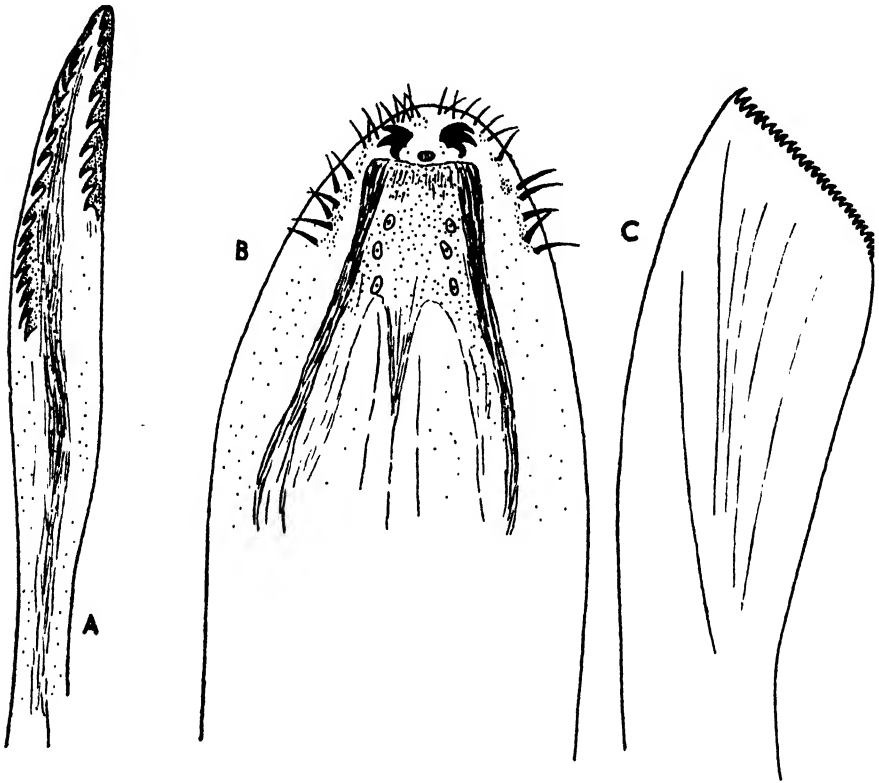


FIG. 3A. *Simulium damnosum*. A.—Maxilla; B.—Labrum epipharynx; C.—Mandible.

larvae it invariably takes up some of them. Corroboration of this view is found in the study of the mouth parts and the ragged type of lesion which the biting parts of this species inflict on the skin.

At the same time the experiments demonstrated the fact that it is not necessary that the larvae of *O. volvulus* should be circulating in the blood in order to permit of their being taken up by a blood-

sucking insect. The repeated examination of the cutaneous blood of these cases during the period proved conclusively that the larvae were very rarely found in a drop of blood taken from the skin ; even on the occasions on which they were found in such a drop it was not feasible to exclude the possibility of their having been derived from the skin ; in fact, that this was the source of those larvae appears highly probable.

Development of O. VOLVULUS larvae in Simulium.

The next step was to endeavour to ascertain whether the *O. volvulus* larvae which were so readily and constantly taken up by *Simulium damnosum* would proceed to develop in the body of this insect provided it was kept alive after feeding on infected skin. Incidentally it would be of interest to discover whether the developmental forms, if found, would correspond to those forms which had already been found in a percentage of the wild flies captured biting on unselected persons. The experiments were as follows :—

(1) Ten flies which had fed on a boy infected in the loin skin, but which were collected from any portion of his body, were kept until they died and were dissected. In one fly which died after thirty hours from the time of feeding, development forms of an early stage were found in the thorax. The other nine, which had all died before three-and-a-half days, proved to be negative.

(2) Large numbers of flies were captured on the two cases (47 and 50) feeding on any part of the body. Of the total of flies so captured very few survived more than a few days. Twenty-two, however, were dissected soon after their deaths and in this total, seven, i.e., 32 per cent., proved to be infected in the thorax.

(3) Twenty-two flies which were captured biting on Case 50, on the band of skin four inches wide, with the nodules in the centre, as described previously, were kept alive. Of these twenty-two flies, which were all dissected at periods later than two days after their feed, eighteen, or 82 per cent., proved to be infected in the thorax. In Table X are set out the percentages of thorax infection obtained in these experiments.

TABLE X.

Showing increase in percentage of thorax infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

| Feeding on | Number of flies dissected | Thorax infected | |
|---|---------------------------|-----------------|------------|
| | | Number | Percentage |
| Unselected boys | 1,320 | 15 | 1'1 |
| One infected boy (all over body) | 10 | 1 | 10'0 |
| Cases 47 and 50 | 22 | 7 | 32'0 |
| Case 50 (nodule band) | 22 | 18 | 82'0 |

From a consideration of these experiments on gut and thorax infection it appeared that when flies were given an opportunity of feeding on known infected skin, the percentage of gut infections rose to a very high figure, such as was never approached by flies captured on casual collectors; again, such flies when kept alive showed a percentage of infection with developing forms in the thorax which was far in excess of anything obtained from the dissection of wild flies fed on casual collectors. In view of the process of exclusion of other larvae, both human and animal, which preceded the last part of this investigation, there appears no doubt that the forms which were developing in the thorax of such flies as fed on the selected cases were derived from larvae ingested from the skin of these cases by the fly and that they were, in fact, the developmental forms of *O. volvulus*.

Examination of other insects.

Reference has already been made to the dissection of the Congo floor maggot. *Glossina palpalis*, which was present but not plentiful in the bush adjacent to the Moendi River, near Tumbudu, was captured on five occasions when feeding on Case 50. Of these five tsetse-flies, two had larvae of *O. volvulus* in the gut, one fly having one and another fly having two. The flies were dissected within an hour of feeding but the larvae were motionless and appeared to be dead. From these few specimens it is evident that *G. palpalis* is capable of taking up the larvae of *O. volvulus*, but it does not appear

that it can take up any numbers comparable with those taken up by *Simulium*. Further, whereas the larvae in the gut of *Simulium damnosum* became more active, the reverse was the case for the larvae discovered in the gut of the two infected tsetse-flies.

Tabanus sp.*

Forty-seven specimens of this fly were dissected in the gut and thorax without the discovery of any larvae. These flies were very common at this place but the natives were seldom bitten by them while they were under observation. The fly is conspicuous not only by its size but by the formidable sound which it makes, and before it settled on the skin the natives were generally prepared for it and dealt with it immediately.

Development of ovaries in Simulium fed on human blood.

The condition found at the time of capture is shown in fig. 4, the ovaries appearing as purely abdominal organs of fusiform shape with small circular ova with a more dense area like a nucleus. When the specimens captured had taken no blood or only a small amount, the ovaries did not undergo development during the next few days.

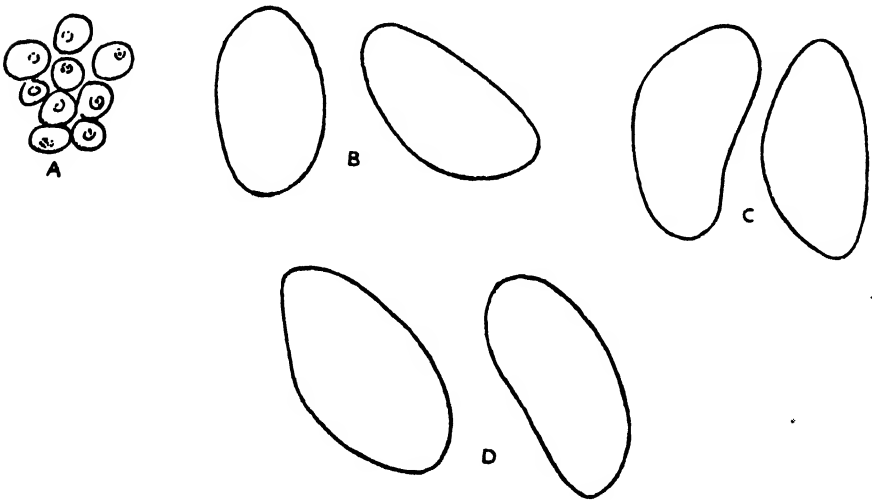


FIG. 4. Ova of *S. damnosum*. A.—Unfed; B.—Fourth day after feed; C.—Fifth day; D.—Seventh day.

* The specimens belong to a new species which will shortly be described by Major E. E. Austen in the *Bulletin of Entomological Research*.

In flies which had fed well the ovarian development was rapid and progressive. The appearance of the ova at various stages is shown in fig. 4. By the fourth or fifth day the ovaries had become partly thoracic in position, so that in separating the thorax from the abdomen a portion of the ovaries and their contained eggs was frequently found in the thoracic region. The increase in size of the ovaries proceeded proportionately to the decrease of blood in the gut so that after a week a fly which had fully fed still had a fully-fed appearance, with this difference, that instead of a globular bulge on each side of the abdominal region, there was a tapering enlargement which diminished as it extended backwards from the thorax to the posterior end of the abdomen.

ACCOUNT OF STAGES

(1) *In subcutaneous nodules.*

The fluid obtained by aspiration of nodules with a syringe contained free larvae, and in several instances where the uterus had been punctured, eggs with embryos in them.

(a) *The egg.*

The egg measured from 46μ to 61μ in length, by 33μ to 51μ in breadth; the egg membrane remained practically unstained when dried, fixed in alcohol and stained with hot haemalum.

(b) *The embryo in the egg.*

These measured from 264μ to 290μ in length, by from 7μ to 9μ in breadth; the cephalic clear area measured from 6μ to 9μ , the caudal clear area from 12μ to 16μ , and the first break from the cephalic extremity was situated at from 21 to 25 per cent. of the length. The nuclei were arranged irregularly in a somewhat scattered manner in the body and were stained to a depth comparable with one group of free larvae in the fluid, but less deeply than another group; these two groups of larvae occurring in the nodules are referred to below in dealing with the free larvae.

(c) *The free larvae.*

The larvae which were found free in the nodule fluid presented two types. The first was a large form very similar, in the scattered

arrangement of the nuclei and in staining reaction, to the form found in the egg; the measurements were, as a rule, slightly greater than those of the egg forms. The second was a small form having a more compact arrangement of the nuclei and taking the stain more deeply than either the large larvae or the egg forms. Although there was some overlapping in measurements and in the density of staining in individual forms, the differences of the two types were as a rule easily observable.

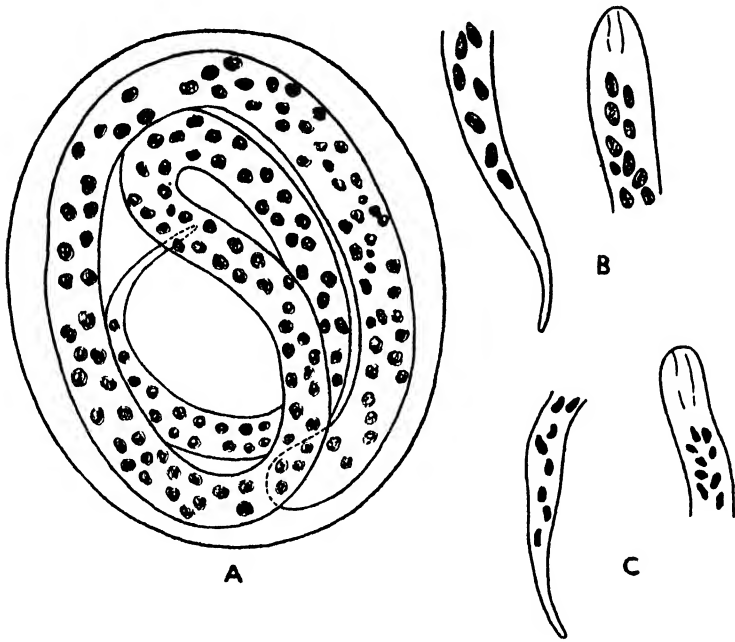


FIG. 5. In puncture fluid from nodule. A.—Larva in egg membrane; B.—Large pale forms of larva; C.—Small deeply stained form.

Large forms.

These varied in length from 295μ to 358μ , and in breadth from 6μ to 9μ ; the cephalic clear area measured from 7μ to 11μ and the caudal from 13μ to 18μ ; the first break from the cephalic end was at from 21 to 25 per cent. of the length; in these forms, as also in the egg forms, owing to the irregular disposition of the nuclei, even the first break was sometimes difficult to determine with accuracy.

Small forms.

These forms usually measured from 221μ to 287μ in length by from 5μ to 7μ in breadth; the cephalic clear area measured from 5μ to 8μ , the caudal clear area from 10μ to 16μ ; the first break was situated at from 22 to 25 per cent. of the length.

(2) *In the skin.*

Great variation in size was evident in the forms derived from the skin. Whereas forms which were measured in fresh preparation varied from 306μ to 322μ in length by from 6μ to 9μ in breadth, specimens in water, which had been allowed to dry on the slide, and then fixed by absolute alcohol, and stained, showed remarkable differences in dimensions. The majority were found to measure from 250μ to 300μ in length; in one case, however, one specimen was found of only 184μ in length and several other specimens in the

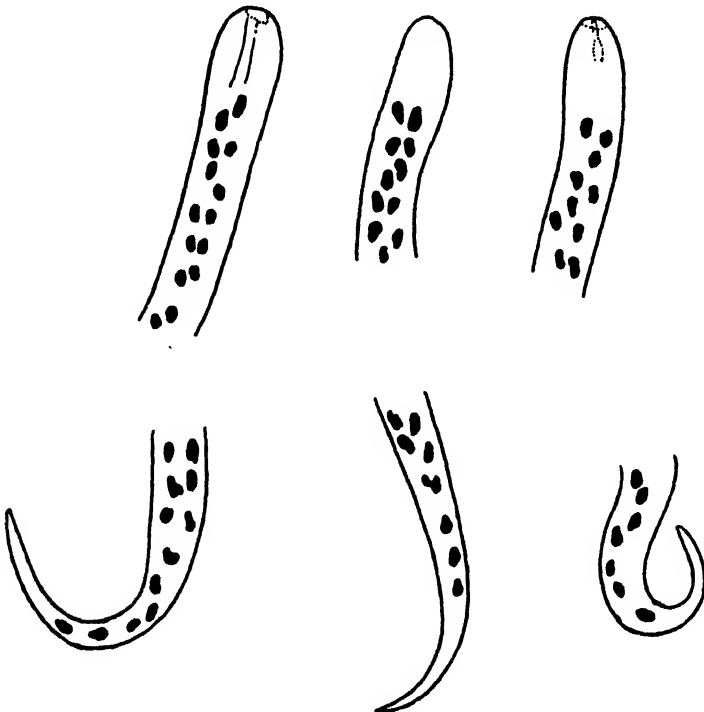


FIG. 6. *O. volvulus* forms in gut of *S. damnosum*.

neighbourhood of 200μ . The larvae in this case were dissected out in water, allowed to dry on the slide, fixed in absolute alcohol and stained by prolonged immersion in dilute carbol methylene blue followed by heated haemalum. In ten specimens measured in this case, the length varied from 184μ to 229μ ; the cephalic clear area measured from 5μ to 9μ and the caudal from 9μ to 21μ ; the first break from the cephalic end occurred at from 20 to 23 per cent. of the length.

The usual breadth of the skin larvae was from 5μ to 9μ , the cephalic clear area measured from 5μ to 13μ , the caudal from 9μ to 21μ ; the first break from the cephalic end was situated at from 21 to 26 per cent. of the length.

(3) *In the gut of Simulium damnosum.*

The forms found in the gut of the fly measured from 200μ to 334μ in length by from 5μ to 8μ in breadth; the cephalic clear area measured from 6μ to 8μ and the caudal clear area from 10μ to 17μ ; the first break from the cephalic end occurred at from 20 to 24 per cent. of the entire length. The measurements are set out in the following table.

TABLE XI.

Giving the measurements of the forms so far dealt with.

| Form | Length μ | Breadth μ | Clear area | | First break at per cent. of length |
|--------------------------|-----------------|------------------|---------------|---------------|--|
| | | | Head μ | Tail μ | |
| 1. Nodule :— | | | | | |
| (a) Egg | 46-61 | 33-51 | ... | ... | ... |
| (b) Larvae in egg ... | 264-290 | 7-9 | 6-9 | 12-16 | 21-25 |
| (c) Free pale forms ... | 295-358 | 6-9 | 7-11 | 13-18 | 22-25 |
| (d) Free dark forms ... | 221-287 | 5-7 | 5-8 | 10-16 | 22-25 |
| 2. Larvae in skin | 184-322 | 5-9 | 5-13 | 9-21 | 20-26 |
| 3. Larvae in fly gut ... | 200-334 | 5-8 | 6-8 | 8-17 | 20-24 |

Movements of larvae.

Within the egg in the nodule fluid only sluggish and partial movements of the larvae were observed. The larvae free in the fluid moved actively but the movement was confined to a coiling and twisting movement without progress across the field. The larvae from the skin when freed in a drop of water on a slide were, as a rule, more sluggish in their movements than the free larvae in the nodule fluid. The chief exceptions to this were the larvae obtained in skin sections from the loin in the case of two adult females; the larvae from these cases showed more active movement than did those from the skin or the subcutaneous nodules in the males examined. In the gut of the fly the larvae proved considerably more active in their movements than in either the nodules or the skin; they exhibited even a certain degree of translatory movement when they were in contact with the blood and débris on the slide.

DEVELOPMENTAL FORMS FOUND IN THE THORAX AND HEAD OF *SIMULIUM*

A. In casual flies.

Certain flies dissected soon after capture contained forms of developing larvae in the thorax and head. The developing larval forms were of various sizes and comprised two very distinct stages, with several intermediate forms.

The two stages were:—(1) Forms of a shape corresponding to those given in fig. 7, with a wide body and definite tail, with or without clear spots in the anterior and posterior portions of the body, and with a more or less developed intestine. A series of such forms ranging in length from 166μ to 425μ , and in breadth from 18μ to 43μ , was collected from the dissection of the thorax of casual flies. (2) Forms usually of a greater length than those and devoid of definite tail. These forms were, as a rule, considerably narrower than the preceding and differed in having a definite intestine and a slit-like anus; a series of such forms collected from the dissection of the thorax of casual flies gave a range of length from 560μ to 660μ , and of breadth from 18μ to 28μ .

Developing forms were not often present in large numbers in those casual flies which were infected; in most cases little movement

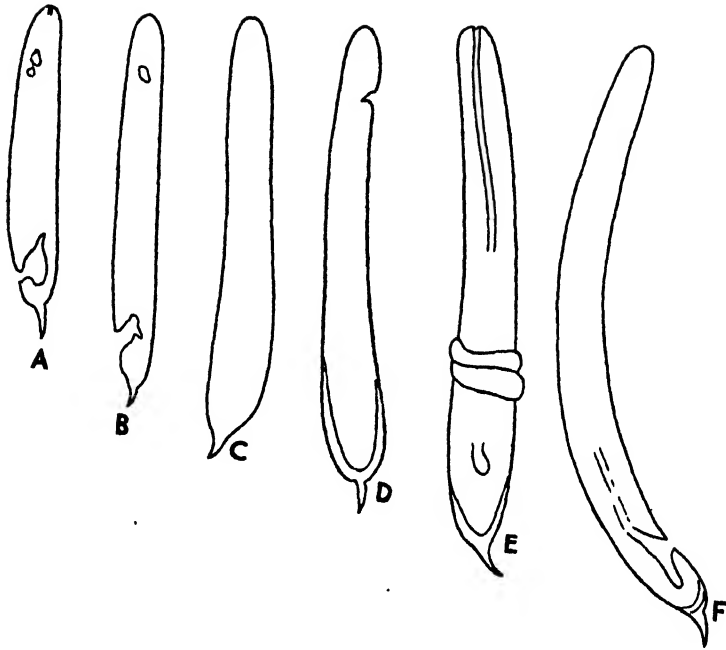


FIG. 7. *O. volvulus* forms with caudal appendage from thorax of wild *Simulium damnosum*. A.— $21.4\mu \times 34\mu$; F.— $425\mu \times 38\mu$. Forms D, E, and F in process of ecdysis.

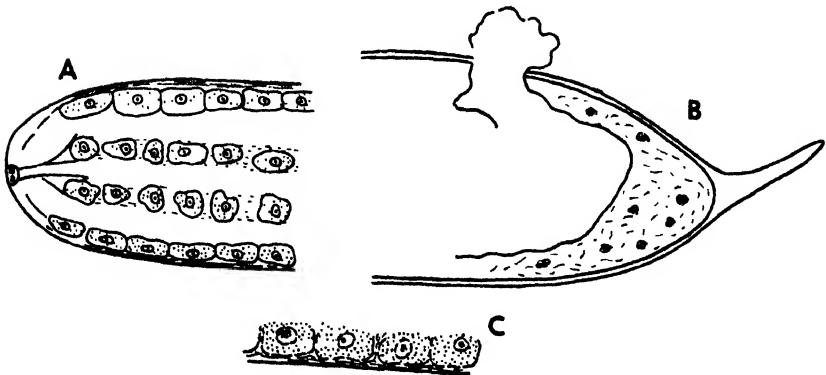


FIG. 7A. *O. volvulus* forms in thorax, wild *Simulium damnosum*, $266\mu \times 33\mu$. A.—Head end; B.—Tail end; C.—Subcuticular cell layer, mid-third of body.

was observed beyond a slight flexion of one or other extremity. Some of the large forms, however, continued to move for a short time after liberation from the thorax. The only form which exhibited very active and prolonged movement came out of the thorax attached to the head on separation of the head from the thorax. It moved very actively and incessantly for a period of three hours, showing very considerable power of penetrating masses of débris on the slide. Unfortunately it was lost in the manipulation of the coverslip. This individual is not included in the measurements given.

B. In flies kept alive after feeding on infected skin.

The forms found in these flies at various dates after being captured feeding on infected human skin were similar in measurements and in morphology to those found in the flies of group A. A series of wide forms possessing a tail varied in length from 173μ to 461μ , and in width from 17μ to 45μ ; a series of forms without a tail measured from 349μ to 628μ in length, and from 20μ to 38μ in width.

Some of the longer forms exhibited active movements, but it was observed that some forms which were active were of smaller size than other inactive forms in the thorax of the same fly. This possibly has a connection with the process of ecdysis.

The numbers of forms dissected out of the thorax in several of the flies which had thus fed on known infected skin was large. In a fly which was dissected sixty hours after thus feeding, no less than 236 developmental forms were recovered from the thorax. In another fly which died on the fifth day sixty forms were found. In a fly which lived until the seventh day ten forms of a much more advanced stage of development were found. It was observed that in some flies there were present forms of different stages. The explanation which naturally suggests itself is that those flies had acquired infection at some period previous to, as well as at the time of, their feeding on the infected case. Such an explanation will not, however, suffice to account for a case such as the following. The flies referred to above which had fed sixty hours previously on infected skin presented, in addition to the early thoracic developing forms, a form which appeared to be at the same stage of development

as the forms just ingested by the fly, and a form of shorter and broader type evidently about midway in development. It was in excellent condition and stained well. The explanation of this probably is either that some of the forms ingested by the fly have not developed sufficiently before their ingestion to enable them to keep pace with the rest, or that they have for some reason failed to reach a suitable portion in which further development can proceed.

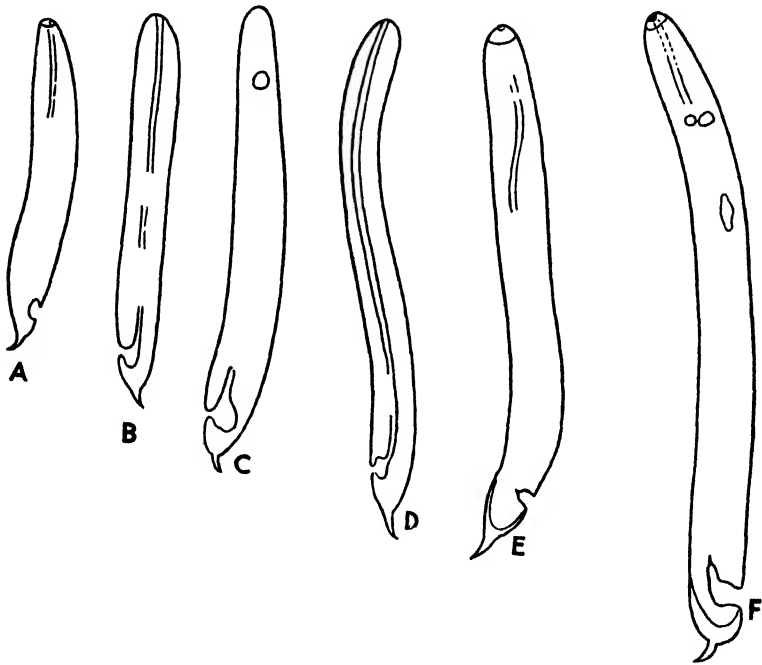


FIG. 8. *O. volvulus* forms with caudal appendage from thorax of *S. damnosum*, five days after feeding on infected skin. A.— $223\mu \times 36\mu$; F.— $440\mu \times 36\mu$. Forms E and F in process of ecdysis.

The argument which is applicable to this case can probably, with justification, be applied to cases at a later date where, in the same fly presumably infected at the time of feeding on known infected skin, there appear, in addition to forms which represent a correct stage of development for the period as judged by the average development in other flies, forms which represent an earlier stage.

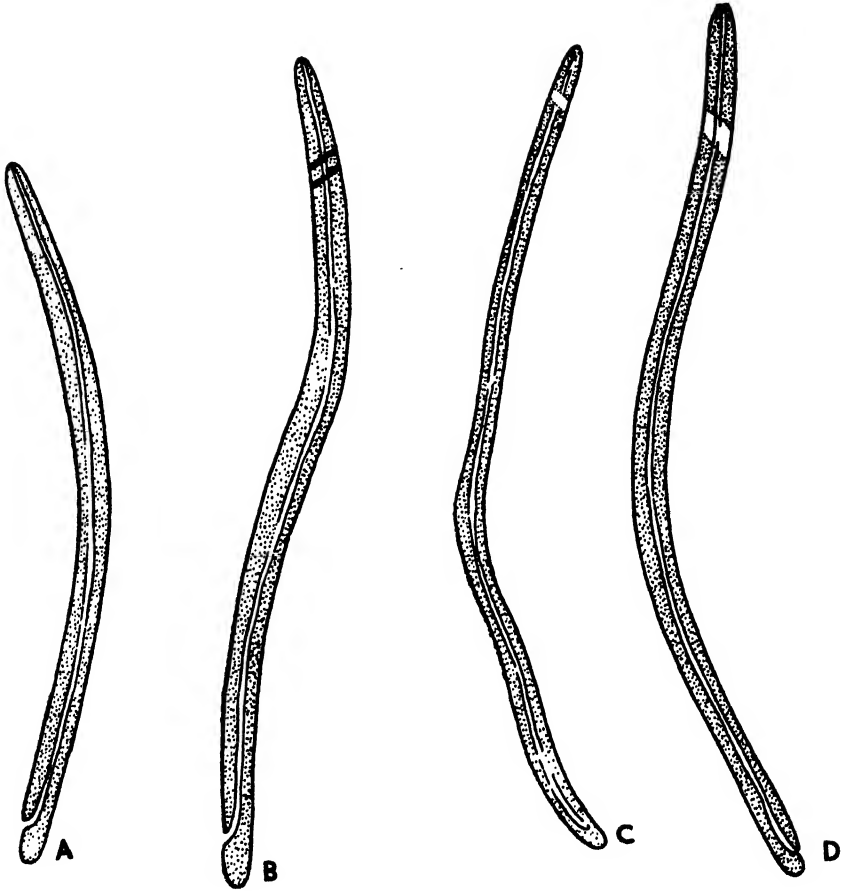


FIG. 9. A.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed. $505\mu \times 25\mu$.
 B.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed. $592\mu \times 32\mu$.
 C.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed. $616\mu \times 25\mu$.
 D.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed. $657\mu \times 28\mu$.

Ecdysis.

It is evident that throughout the process of development of the larva several changes are brought about which could most readily be explained on the basis of ecdysis. If this is not the explanation it is difficult to understand how, at various stages, the larva undergoes the considerable variations in length and breadth observed. Thus in the tumour fluid the large pale form which resembles the egg form is associated with a smaller and darker-stained form with compact nuclei; in the skin, only forms corresponding to the latter type were found, but again with a diminution in length of some individuals. In the thorax the shortest form found in any fly measured only

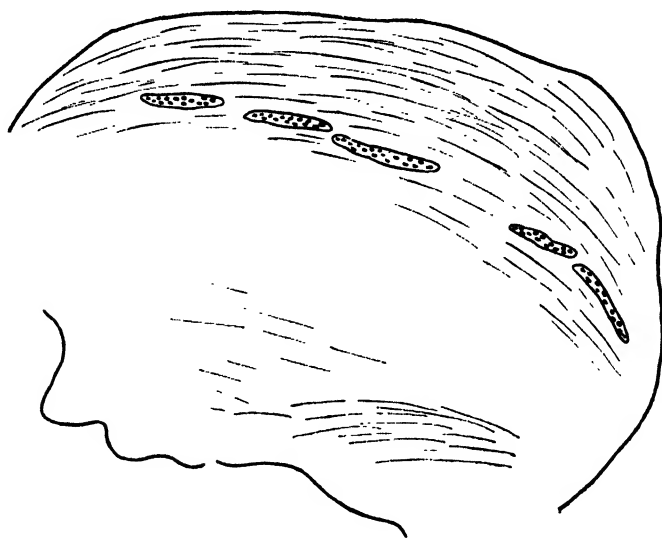


FIG. 10. Section of thorax of *S. damnosum* on fifth day after feeding on infected skin, showing portions of developing forms of *O. volvulus* larvae.

166 μ , yet in the skin of infected persons the shortest form found was 184 μ , while in the gut of infected flies the shortest form found was 200 μ . What seems to be actual ecdysis was only observed in the case of the transition from the tailed thorax form to the thoracic form without tail; it was observed that in some cases the active forms later developed in the thorax were accompanied by forms slightly longer and immobile, and the appearances suggested that at this stage also, an ecdysis had occurred from the more elongated immobile form to the active form.

SUMMARY

1. *Onchocerca volvulus* infection is common in the Konno District of the Protectorate of Sierra Leone ; larvae of this parasite were found in the skin of 45 per cent. of persons examined systematically.

2. A definite relationship between diseases of the skin or diseases of the eyes and the infection with *O. volvulus* as judged by the presence of larvae in the skin could not be established.

3. *Simulium damnosum* is very prevalent in the hilly country, which is covered with bush and grass, and has numerous streams and rivers ; the lesion inflicted on the skin by this species in biting is such as would dislodge the larvae of *O. volvulus* in the skin.

4. Dissection of wild insects showed :—

(a) In 780 dissections of the gut an infection of 2·6 per cent. with larvae morphologically identical with those of *O. volvulus*.

(b) In 1,320 dissections of the thorax a larval infection of over 1 per cent.

5. By allowing wild flies to feed on restricted and heavily-infected areas of the skin the gut infection was raised to 80 per cent. in one experiment and the thorax infection to nearly 82 per cent. in another experiment ; the developing forms of *O. volvulus* were found in the thorax after the infecting feed up to the seventh, eighth, and tenth days, after which period no insect survived.

PLATE I

EXPLANATION OF PLATE I

- Fig. 1. Nodules on both trochanters.
- Fig. 2. Nodules on ribs (1), and trochanter (2). Puncture positive in both.
- Fig. 3. Painful nodules and a much wrinkled skin.
- Fig. 4. Case 59. Nodules on right elbow (1), and right trochanter (2).



1.



2.



3.



4.

EXPLANATION OF PLATE II

- Fig. 1. Case 59. Nodules on left elbow (1), left trochanter (2), and left knee (1).
- Fig. 2. Case 59. Nodules (near view, right side) on elbow (1), and trochanter (2.)
- Fig. 3. Capturing *S. damnosum*.
- Fig. 4. Type of country infested by *S. damnosum*.



1.



2.



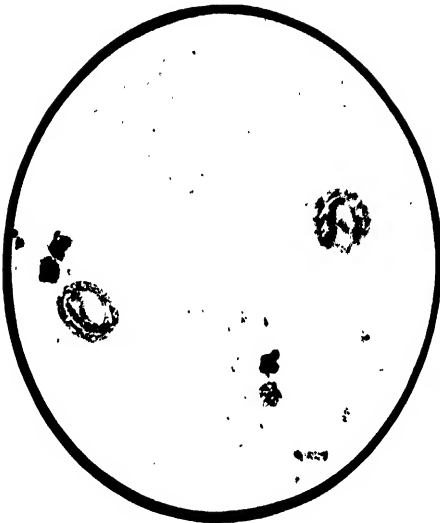
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4.

EXPLANATION OF PLATE III.

- Fig. 1. Micro-photograph showing two eggs of *O. volvulus* in puncture fluid from a subcutaneous nodule. \times about 200.
- Fig. 2. Micro-photograph of larva of *O. volvulus* in puncture fluid from a subcutaneous nodule. \times 200.
- Fig. 3. Micro-photograph of two larvae of *O. volvulus* from skin. \times 200.
- Fig. 4. Micro-photograph of larvae of *O. volvulus* from gut of *S. damnosum*. \times 200.



1.



2.



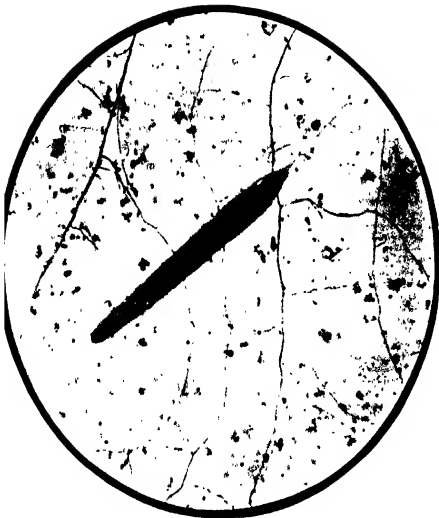
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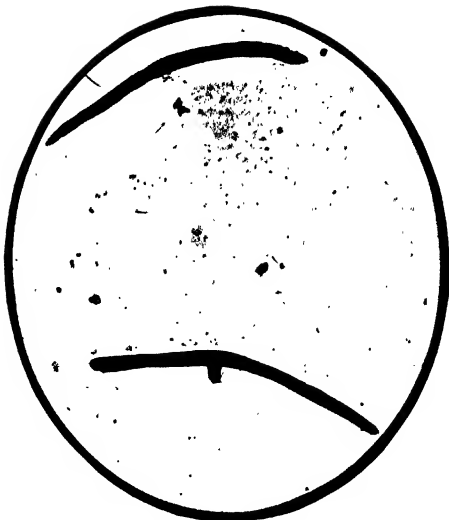
4.

EXPLANATION OF PLATE IV

- Fig. 1. Micro-photograph of larva of *O. volvulus* from thorax of *S. damnosum* sixty hours after ingestion. $\times 200$.
- Fig. 2. Micro-photograph of two larvae of *O. volvulus* from thorax of *S. damnosum* seven days after ingestion. $\times 125$.
- Fig. 3. Micro-photograph of section of skin showing larvae of *O. volvulus*. $\times 200$.
- Fig. 4. Micro-photograph showing skin lesion produced by the bite of *S. damnosum*. $\times 20$.



1.



2.



3.



4.

OBSERVATIONS ON THE DEVELOPMENT OF HOOKWORM LARVAE

PART I

BY

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Neish (1913), in Jamaica, stated that ova and *Ancylostome* larvae were destroyed by normal saline, and on these grounds he explained the absence of hookworm infection on two estates in Portland, where the labourers' barracks were situated on the sea shore. Caldwell (1922), working in Honduras, suggests that the relatively low infection rate among the inhabitants of villages on the shore compared with that found in villages further inland is due to the lethal action of sea water on hookworm larvae. On the other hand, Nicoll (1917) found that even with a 6 per cent. solution of salt, several eggs were capable of hatching after as long as six or seven days in the salt. Nicoll's results suggest that it is something besides the mere presence of salt which prevents the development of hookworm eggs and larvae, and the following series of experiments were undertaken with a view to finding out, if possible, what was the real reason underlying the observations of Neish and Caldwell.

As Maplestone (1924) has shown, *Necator americanus* is much commoner than *Ancylostoma duodenale* in Freetown, and the following results should be taken as applying to the former species. Artificial cultures were used throughout, for although the soil in and around Freetown must be heavily infected with the larvae of *N. americanus*, the results would be unreliable if naturally infected soil were used, because the numerous cats and dogs present being heavily infected with *A. caninum* and *A. ceylanicum*, these larvae would be found in large numbers as well, and Stekhoven and Stekhoven-Meyer (1924) have shown that the larvae of these two species are indistinguishable from those of the worms infecting human beings. In this connection,

the writer is not aware that *A. caninum* and *A. ceylanicum* are present in the Islands of Trinidad and Porto Rico, but it is a remarkable fact that in the numerous papers of the series 'Investigations on the Control of Hookworm Disease,' recently published by Cort and many other American workers in these places, the possibility of confusing the larvae of *N. americanus* with those of the above species is not referred to.

TECHNIQUE

All the work was done in the laboratory at room temperature. The cultures were placed on a bench beneath large windows and were covered with glass bell jars ; they thus received plenty of light but were never in direct sunlight.

Except in a few special cases cultures were always examined on the seventh day of growth, so that comparison between them would be as accurate as possible.

The materials used in the cultures were powdered wood charcoal, laterite, which is the natural soil in Freetown, and sand taken from the sea shore below high-tide level. The laterite and sand were heated in enamel saucepans to between 60 and 70 degrees Centigrade for about twenty minutes. This temperature did not dry or char the material and was always found sufficient to destroy all nematodes, whether larvae or adults, that were in the soil. They were then passed through a sieve with a mesh of 4 mm., to remove the larger stones. The materials were stored in tins, and in a few days the laterite became perfectly dry, whereas the sand remained moist for months. The sand was found to be slightly alkaline (pH 7.6) and the laterite was neutral in reaction. The relative weights of charcoal, laterite and sand were 2, 3, and 9, respectively per volume, therefore quantities for making cultures were measured by volume and not by weight, so that all cultures were of equal size and the mixture of faeces and culture medium were in the same proportions on all occasions. The amount of faeces used in a culture was always 3 grams. It was soon found that charcoal mixed with laterite was no better than plain laterite as a culture medium, so that the charcoal was discontinued.

Wire gauze with a mesh of 1.158 mm. was made into small

baskets measuring 4 cm. square and 2 cm. in depth, thus having a capacity of 32 c.c. Small cultures, consisting as they did of 25 c.c. of earth and 3 grams of faeces, easily fitted into these baskets. For isolation of larvae from the cultures the baskets, as a whole, were placed in the isolation apparatus. The advantages of this method were that handling of cultures was simple and rapid, and there was no danger of loss of any of the culture by transferring it to a fresh sieve; the wire gauze also allowed water to drain away freely, so the cultures were never standing in excess water, being unduly waterlogged in consequence. For some cultures on a larger scale that it was found advisable to make, wooden boxes in the shape of cubes, 10 cm. \times 10 cm., were made.

For isolation of larvae from cultures, funnels of suitable size for the culture concerned were fitted with a short piece of rubber tubing closed by a spring clip, in every way similar to the apparatus used by Cort *et al.* (1922). The funnels were lined with a double layer of plain white muslin as it was found that a single layer let too much soil through. The method of dealing with the small cultures has already been described; for the large cultures wire gauze sieves of suitable size were made, using gauze of the same mesh as that from which the small baskets were made. The cultures in the latter case had to be transferred from the boxes to the sieves. After being placed in a suitably sized funnel and the spring clip being applied to the rubber tubing, water at a temperature of 50° C. was poured in until the soil was partly submerged. By starting with water at 50° C. it was reduced to about 45° C. by the time it had come in contact with the culture; this temperature has been shown by Cort *et al.* (1922) to be the optimum for the isolation of larvae, and a few preliminary experiments confirmed this. The water was poured carefully down the side of the funnel, avoiding contact with the culture, until it rose sufficiently high to reach the soil from below. A series of cultures was always put up for isolation in the afternoon and examined on the following morning, that is, fifteen to eighteen hours afterwards.

Many cultures produced thousands of larvae, which rendered counting all of them impossible, so the following method was adopted. Centrifuge tubes holding about 15 c.c. were marked at 10 c.c. and 5 c.c. The clip at the end of the rubber tube was slowly

released, allowing exactly 10 c.c. of water from the culture to flow into the tube. This amount of water was found to contain all the larvae that had come out of the culture. The tube was then centrifuged to throw all the larvae to the bottom and then 5 c.c. of water were drawn off, leaving all the larvae from a given culture in 5 c.c. of water. A Wassermann pipette, cut short to facilitate handling, was fitted with a rubber teat, and this was used for collecting samples for counting. An even suspension of the larvae was first made by drawing up and forcibly expelling portions of the contents of the tube; using the pipette for the purpose, it could easily be determined by the naked eye when this had taken place. The pipette was now filled and 0.1 c.c. allowed to run on to a slide. This operation was repeated five times and five slides from each culture were prepared; but after the taking of each 0.1 c.c. sample, care was taken to mix the suspension of larvae thoroughly, because they were found to settle to the bottom very rapidly. The number of larvae in each of the five drops was counted, the five totals added, and this figure being multiplied by ten gave the number of larvae in the original 5 c.c. of water, that is, the number of larvae isolated from the culture. A cover slip was found unnecessary as the larvae could be counted easily with a low power (Zeiss objective A, and eyepiece 4). In counting, a mechanical stage was used and the whole drop of water passed under review; to avoid counting larvae more than once or missing them altogether, those larvae that only partly showed in the lower edge of the field were counted and those that only partly showed in the upper edge of the field were omitted. A drop of Lugol's solution was added before counting because it rendered the larvae motionless at once; this was found necessary for, with a large number of larvae wriggling actively about, it is not possible to count them accurately, and a further possible source of error is that some larvae may pass out of the field either before or after being counted, and the same larvae might thus be encountered in another part of the drop. Another advantage of the use of Lugol's solution is that it enabled the distinction between hookworm and *Strongyloides* larvae to be made rapidly and accurately; this is a simple matter in the ordinary way, but when there are large numbers of larvae wriggling about and some of the hookworm larvae have lost their sheaths, it is not so easy to distinguish between them.

In the following experiments it will be noted that the numbers of larvae isolated in different series of cultures are very different ; this is because faeces from many individuals were used during the course of the work.

For the sake of brevity all the experiments given below are expressed as a single figure which, however, represents the average of a large number of individual experiments.

SECTION I. The Effect of Sea Water and Sea Sand on the Development of Hookworm Eggs and Larvae.

EXPERIMENT I.

Portions of faeces 3 grams in weight were mixed with laterite and charcoal, half of the cultures were kept moist with sea water, and half with tap water as controls.

Cultures moistened with sea water produced 313 larvae per gram of faeces, and the controls moistened with tap water produced 328 larvae per gram of faeces.

This clearly shows that sea water alone has no effect on the development of hookworm eggs and larvae.

EXPERIMENT 2.

The effect of sea sand was next tried, being controlled by cultures of faeces in charcoal and laterite mixture, and all cultures were moistened with tap water. Sand and laterite prepared in the manner described were used throughout. In a series of over twenty different experiments on these lines, using faeces from several individuals, the number of larvae per gram of faeces recovered from sand cultures varied from nil to about 20 per cent. of the numbers recovered from the charcoal-laterite controls.

Next, a series of seven cultures in sand and a similar number of controls in charcoal-laterite mixture were put up. One each of these cultures was examined every day from the first to the seventh day of growth, and it was found that on the first and second days the sand cultures and the controls produced an approximately equal number of larvae ; but from the third day onwards the numbers in the sand cultures gradually diminished, whilst the controls remained unaltered. This clearly indicated that, whatever the action of the sand might be, it was exerted on the young larvae and not on the eggs before hatching.

A portion of previously heated sea sand was washed by allowing tap water to percolate through it for twenty-four hours. It was then dried and used as a culture medium, controlled by cultures in charcoal-laterite and unwashed sea sand. In addition to mixing with sand alone, various modifications such as mixing faeces with sand-charcoal-laterite in various proportions or covering cultures with a layer of one or other of the media, were also tried. Three grams of faeces were used in all cases, the cultures were grown for seven days, and the results are expressed in terms of the number of larvae isolated from one gram of faeces. In the following series of experiments unwashed sea sand is called 'sea sand,' washed sea sand is called 'washed sand,' and charcoal-laterite mixture is called 'earth.'

EXPERIMENT 2a.

| | |
|--|---------------|
| Faeces-earth-washed sand in equal parts produced | 400 larvae. |
| Faeces-earth-sea sand in equal parts produced | 1,440 larvae. |
| Faeces-earth, covered with an equal volume of washed sand produced | 530 larvae. |
| Faeces-earth, covered with an equal volume of sea sand produced | 90 larvae. |

EXPERIMENT 2b.

| | |
|------------------------------------|-------------|
| Faeces-washed sand produced | 143 larvae. |
| Faeces-sea sand produced | 27 larvae. |

EXPERIMENT 2c.

| | |
|---|-------------|
| Faeces-earth not covered produced | 295 larvae. |
| Faeces-earth covered with washed sand produced | 108 larvae. |
| Faeces-earth covered with sea sand produced | 79 larvae. |
| Faeces-washed sand not covered produced | 102 larvae. |
| Faeces-sea sand not covered produced | 10 larvae. |

EXPERIMENT 2d.

| | |
|--|-------------|
| Faeces-earth not covered produced | 920 larvae. |
| Faeces-earth covered with an equal volume of same produced ... | 957 larvae. |
| Faeces-earth covered with an equal volume of washed sand produced | 727 larvae. |
| Faeces-earth covered with an equal volume of sea sand produced ... | 100 larvae. |

EXPERIMENT 2e.

| | |
|--|-------------|
| Faeces-earth produced | 860 larvae. |
| Faeces-earth (1 part)-sea sand (1 part) produced | 27 larvae. |
| Faeces-earth (1 part)-sea sand (2 parts) produced | 37 larvae. |
| Faeces-sea sand produced | 0 larvae. |

The above series of experiments indicates that sea sand has a lethal effect on hookworm larvae when used under the conditions of these experiments, and they show that unwashed sand is more powerful than washed sand. It was now decided to test the same materials, using the same amount of faeces but much larger amounts of earth and sand, and for this purpose wooden boxes 10 cm. square by 10 cm. in depth were employed in place of the small wire gauze baskets. The boxes were filled with laterite to within one inch of the top, the faeces was placed on the surface and covered with a thin layer of the material to be tested. From now on charcoal-laterite mixtures were replaced by plain laterite as it was found that the latter was a slightly more favourable culture medium.

EXPERIMENT 2f.

| | |
|--|--------------------|
| Faeces-earth covered with laterite half-inch and moistened daily | |
| produced | 446 larvae. |
| Faeces-earth covered with sea sand half-inch and not moistened | |
| produced | 483 larvae. |
| Faeces-earth covered with sea sand half-inch and moistened daily | |
| produced | 518 larvae. |

This series of experiments indicates that sea sand seems to be effective only in small cultures and not in large ones ; this is difficult to understand as, with a much greater proportion of sand to faeces, it might be expected that the effect would be more marked in large cultures than in small ones. As a check on this contradictory result a fresh series of small cultures was made in the wire baskets.

EXPERIMENT 2g.

| | |
|---|--------------------|
| Faeces-earth covered with earth produced | 382 larvae. |
| Faeces-earth covered with sea sand produced | 346 larvae. |

This apparently showed that the sand had lost its former effect, and as the sand used up to this time had been stored in the laboratory for some months it was thought this might be the reason. Accordingly a fresh lot of sand was procured and after being heated in the same way as the first lot it was tried under identical conditions.

EXPERIMENT 2h.

| | |
|---|----------------------|
| Faeces-earth covered with earth produced | 5,612 larvae. |
| Faeces-earth covered with old sand produced | 5,042 larvae. |
| Faeces-earth covered with new sand produced | 3,050 larvae. |

This showed that the new sand was more effective than the old sand, but it was not nearly so powerful as the old was when first collected.

EXPERIMENT 2j.

Testing the new sand in larger quantities, viz., in boxes.

| | | | | | |
|---|-----|-----|-----|-----|---------------|
| Faeces-earth covered with earth produced | ... | ... | ... | ... | 1,327 larvae. |
| Faeces-earth covered with new sand produced | ... | ... | ... | ... | 933 larvae. |

Reduction of the results of the two experiments 2h and 2j, to the same proportion, gives 1.5 : 1.47, which shows that the effect of the new sand is practically the same in small and large cultures. But in less than a week it was found that the new sand was as ineffective as the old.

All the experiments hitherto recorded have been subjected to the same conditions of moisture except where the contrary is stated, and the cultures in any given series have always been wetted with equal amounts of water and at the same time.

The foregoing experiments were carried out from August to the beginning of November, at which time the sand was found to have become ineffective. The reason for the loss of power in the sand was puzzling until it was realised that the change in its efficacy coincided with the change from the wet to the dry season. During the rains in Sierra Leone, humidity is high and evaporation is, consequently, slight. It had been noted that sand cultures were always more sodden with water than corresponding earth cultures, even when equal amounts of water were used for moistening them, suggesting that earth is able to absorb more water than sand. The idea, therefore, suggested itself that the suspected lethal effect was not in the sand *per se* but was due to the inability of the sand to absorb water as readily as earth, with consequent excess of water in the former type of cultures. This excess was more marked in the wet than in the dry seasons for, during the latter, the greater evaporation would allow the sand cultures to approximate more nearly to the earth cultures with respect to the amount of free water present. This hypothesis was supported by the observation that, in boxes which contained a large quantity of earth below the cultures, the sand was not effective at all, and this could be explained as follows :—The large quantity of absorbent earth beneath the sand drained excess of water out of the latter, thus allowing the sand to become a suitable culture medium.

The next series of experiments was devised with the object of testing the accuracy of the above theory. Two cultures were put up

in sand and two in laterite, one of each being used as controls. In this case the controls had only enough water added to keep them just moist, the sand control requiring much less water than the laterite control. The other cultures were freely moistened daily so that they were always in a state of saturation. This experiment was repeated six times and the results are expressed as an average of the whole.

EXPERIMENT 3a.

| | | | | | |
|---|-----|-----|-----|-----|-------------|
| Faeces-laterite kept just moist produced | ... | ... | ... | ... | 303 larvae. |
| Faeces-sand kept just moist produced | ... | ... | ... | ... | 300 larvae. |
| Faeces-laterite freely moistened produced | ... | ... | ... | ... | 117 larvae. |
| Faeces-sand freely moistened produced | ... | ... | ... | ... | 120 larvae. |

This clearly shows that sea sand and earth, when under equal conditions of moisture, are of the same value as culture media, and as a corollary of this it is evident that, when moistened with equal quantities of water, sand appears a worse medium than laterite because of its inability to absorb water as readily as the latter. Further confirmation of this was obtained by the following series of experiments. They were devised with the object of obtaining as nearly as possible the condition of faeces lying on the ground in the water-logged soil at the edge of a stream or pond.

EXPERIMENT 3b.

Small glass jars about 15 cm. in height and 3 cm. in diameter were filled with laterite to within about 4 cm. of the top. Portions of faeces, 3 grams in weight, were mixed with 25 c.c. of laterite and placed on top of the laterite in the jars. The surface of the culture was not flat but was sloped upwards to one side of the jar, so as to reach nearly to the top. Water was now poured into the jar until it just reached the junction of the original soil and the culture, and it was kept at this level continuously by making good every day any loss from evaporation. The effect of this was that the cultures were always completely saturated with water but were not immersed in it. A similar set of jars was prepared, using sea sand instead of laterite, and sea water instead of tap water. For examination the cultures were carefully removed from the surface of the original soil in the jars and were placed in wire baskets for isolation in the usual way. The cultures were then drained off, allowed to remain in the baskets for another week, being kept just moist meanwhile, and

they were then tested by isolation a second time. Two series of cultures were prepared, one being kept in the jars for a week and the other for two weeks before isolation.

SERIES 1 (kept for seven days).

| | | | | | |
|------------------------------|-----|-----|-----|-------------|------------------|
| Laterite culture produced... | ... | ... | ... | 50 larvae. | } 1st isolation. |
| Sand culture produced | ... | ... | ... | 7 larvae. | |
| Laterite culture produced... | ... | ... | ... | 67 larvae. | } 2nd isolation. |
| Sand culture produced | ... | ... | ... | 197 larvae. | |

SERIES 2 (kept for fourteen days).

| | | | | | |
|------------------------------|-----|-----|-----|-------------|------------------|
| Laterite culture produced... | ... | ... | ... | 230 larvae. | } 1st isolation. |
| Sand culture produced | ... | ... | ... | 20 larvae. | |
| Laterite culture produced... | ... | ... | ... | 0 larvae. | } 2nd isolation. |
| Sand culture produced | ... | ... | ... | 223 larvae. | |

Control cultures in gauze baskets produced 820 larvae, and as the residual soil in the jars did not contain any larvae it is clear that saturation of soil containing faeces is sufficient to reduce greatly the number of larvae that will develop from it even if brought into more favourable surroundings later on. Payne (1922) has noted the fact that the death rate of hookworm larvae is high in water-logged soil, and the foregoing experiments seem to be a clear confirmation of this.

CONCLUSION

The evidence of the foregoing experiments tends to show that sea water and sea sand *per se* have no effect in preventing the development of hookworm eggs and larvae.

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BREEDING PLACES OF ANOPHELINE MOSQUITOS IN AND AROUND FREETOWN, SIERRA LEONE

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PLATE V AND MAP

The survey, details of which are given in the present paper, was undertaken during the period 26 May to 9 September, 1925, that is, at the commencement and during part of the rainy season.

We are indebted to H. O'Hara May, Esq., M.D., Deputy Director of Sanitary Services, and to the officials of the Sanitary Department, for much valuable assistance which they rendered throughout the period of the investigation.

At various times during the last twenty-five years, investigations have been made by different observers as to the prevalence and distribution of mosquitos in Freetown. During this period there have been great and progressive reductions in the number of mosquitos in general. The findings of Ross, Annett and Austen (1900), and Stephens and Christophers (1900), who examined the town and its environs for mosquitos, prove that the anophelines were present twenty-five years ago, in numbers vastly greater than could be found by any subsequent observers. It has, in fact, frequently been recorded by the latter that in Freetown itself, at any rate, mosquitos are by no means plentiful and rarely obtrude themselves in such numbers as to constitute a cause of physical discomfort. Thus Bacot (1915) writes of the apparent absence and actual rarity of mosquitos in Freetown. Blacklock (1921), who made a survey of anopheline breeding places in the town at the end of the dry season, found

anophelines practically entirely confined to their dry season residual breeding places, which were the pools in the courses of the streams which traverse the town and chiefly at the points above and below the area occupied by streets.

The present detailed survey has included not only the town portion but also, in some cases, the hilly portions of these streams far beyond the town boundaries. A number of species of *Anopheles* have been found, which have either not previously been recorded from the neighbourhood of Freetown or have been known only from a few specimens.

A plan of Freetown, issued by the Public Works Department, Sierra Leone, 1911, is reproduced in part, reduced to about two-fifths and slightly altered, at the end of this report.

The following species were found :—*

1. *Anopheles costalis* Theo.†
2. *A. funestus* Giles.
3. *A. rhodesiensis* Theo.
4. *A. smithii* Theo.
5. *A. marshalli* var. *freetownensis* Evans.
6. *A. squamosus* Theo.
7. *A. theileri* Edw.
8. *A. domicolus* Edw.

THE DISTRIBUTION AND BREEDING PLACES OF THE SPECIES OF ANOPHELES FOUND

A. costalis Theo.

At the beginning of the investigation this species was found breeding in the stream which opens at Magazine Wharf (see Map), in Nicol's Brook, in Sander's Brook and in the Alligator River. The breeding places were not evenly distributed along the courses of these streams; in the first two the most prolific breeding places were in the portions of the stream below the town, extending from the streets to the sea. Through the town there were often long stretches which proved entirely free from larvae, but they were found again in numbers at the outskirts of the town.

* All these species were determined from adults bred from the larvae or pupae.

† Christophers (1924) has shewn that the correct name for this species is *Anopheles gambiae* Giles.

There are several points of interest which may be referred to in detail. For example, the stream which opens at Magazine Wharf is, until just before its entrance to the sea, contained in a laterite drain, and here its flow is fairly rapid ; there is little or no vegetation and the water is used for washing clothes at various points. Throughout this portion no larvae of *Anopheles* were present. After it passes the railway, however, the stream bed widens out into a natural bed of a rocky and steep nature ; the pools found here contained abundant larvae, even to within a few yards of the salt water. Again, in Sander's Brook a notable feature was the regular occurrence of the breeding places at those points at which a lateral drain from the streets joined the main stream. In a stretch of stream running through the town are nine such junction drains ; at six of these, larvae were found in the semi-stagnant weedy area which existed near the junction ; they were not found in large numbers in the stream itself at these points. Certain negative findings are worth recording, e.g., no larvae of this species were found in Granville Brook, skirting the east of the town or, during July and August, in the top of Sander's Brook, above Mendi Street, where the water course has been trained so that the stream flows between loose banks of laterite stones. In September, however, larvae were taken at the latter place in the area of overflow caused by the heavy rains. Isolated breeding sites were found away from the streams in the areas adjacent to them, usually in small seepage waters or in laterite drains, especially on the east side near the outskirts of the town.

In addition to breeding places in these natural and semi-natural waters, there were breeding places of this species in such sites as the following :—

1. Pools in laterite street drains.
2. A concrete washing tank (Pl. V, fig. 4).
3. Canoes lying on the beach at various points.
4. Shallow pits dug in laterite and containing clean water or, as in one case, foul water with an accumulation of decomposing vegetable refuse.

Outside the town, towards the hills behind it, this species was only rarely found in tracing the streams to their source in the hills. The upper part of Nicol's Brook outside the town is rocky, steep

and well shaded by trees ; here *A. costalis* was replaced by *A. smithii*. It was remarkable to observe the suddenness with which the latter replaced the former species just beyond the town.

Three collections of larvae taken from water lying in exposed, flat stones, in compounds at Hill Station, were sent to us by the Sanitary Department. None of these were larvae of *A. costalis*.

In general, it may be said that in Freetown *A. costalis* breeds most freely in pools at the edges of slowly running portions of the streams ; these pools may or may not be isolated from the current. It is often difficult to say whether a pool is actually separated from the current, but it is frequently possible by stirring up the mud or sand to observe that there is a current of water circulating through pools that are apparently isolated from the stream. While vegetation is usually present in sites containing large numbers of larvae, numerous larvae have been found in water apparently free from vegetation. *The breeding places, almost invariably, are so situated as to receive a considerable amount of sunlight.* The bottom of the pool as well as the sides may be mud, rock, sand or gravel. The character of the water varied from perfectly clear to discoloured and, in one case, foul with rotting vegetation.

If the discovery of large numbers of eggs indicates a preferential breeding place, the following observation from Nicol's Brook may be of interest. Three pools of similar character yielded hundreds of eggs ; they were small, shallow pools each containing about a gallon of water, situated beside rocks in the bed of the stream. In each case the bottom was gravelly, no vegetation was growing actually in the water but low herbaceous plants overhung it on one side ; no shade was present except the small amount afforded by this vegetation and the adjacent rocks.

We did not find this species breeding in small artificial containers such as tins, bottles, native pots, nor in rot-holes in trees, nor in the water contained in the axils of such plants as *Dracaena* (the 'cocked-hat tree').

From the earliest periods of investigation of the anophelines in Freetown it has been evident that *A. costalis* has been the prevailing species breeding in the town. This was the experience of Ross, Annett and Austen (1900), Daniels (1901), Logan Taylor

(1902), and also in more recent times that of Bacot (1915) and Blacklock (1921) ; the last-named wrote ' This (*A. costalis*) appears at the present time to be by far the commonest Anopheline breeding at the end of the dry season in Freetown.' Comparing our findings of to-day (see Map) with those of the earliest observers we may note that while it is clear that there has been an enormous reduction of adults and breeding places since their time, it is equally clear that the numerical predominance of this species still remains. In Freetown, itself, therefore, this important carrier of malaria is the Anopheline, above all others, which has to be reckoned with. It will remain a standing menace certainly until such time as the streams through the town are treated in a radical manner.

Anopheles funestus Giles.

This species was only once found by us in the town itself ; we obtained it, however, in two localities adjacent to the town. The breeding site in the town was in Elliott Street in the western area ; four fully-grown larvae being found in water in a small, unshaded pit in laterite into which dead sticks had been thrown ; numerous larvae of *A. costalis* were taken at this spot at the same time and on other occasions. Outside the town one site was in a garden at the point where the road to Kissy crosses Granville Brook ; the larvae were found in a shallow stream of water rapidly trickling among syenite rocks, on dead leaves, with a small amount of vegetation and exposed to the morning sun. The other site was on the portion of Alligator River just above the town (Pl. V, fig. 3) ; they were found in small numbers over a stretch of a hundred yards of river well above the highest breeding place of *A. costalis* found by us on this stream, and separated from it by a stretch of water in which no larvae were found. The larvae at this place were found among weeds growing at the margin of fairly rapidly running water ; the weeds were growing in water that was more than a foot deep in most places ; there was a little tree shade and the sides and bottom of the stream were of soft mud.

It will be observed that these three breeding places were widely different in character.

Ross, Annett and Austen (1900) found *A. funestus* restricted entirely to the eastern part of the town : ' We never found a larva

or an adult of this species west of Government House Individuals were caught in a house in the town where some cases of fever had occurred at Leicester, a village high among the hills, and at some other points. The larvae were common in pools in the eastern quarters, at Kissy and other spots.' Daniels, quoted by Ross (1901), noted that *A. funestus* was found near but not in Freetown. Bacot (1915) records *A. funestus* only from outside Freetown at the following places :—Hill Station, Kissy Flats, and the area between Kissy Road and Fourah Bay. Blacklock (1921) also failed to find *A. funestus* in Freetown.

Concerning the breeding places of this species, Logan Taylor (1902) says that it seems to prefer gently running water to breed in, the larvae being found where the currents are sluggish, and especially amongst grass and weeds at the edge. Two of the sites discovered by us agree in most respects with this writer's observations with regard to the character of the breeding places of this species. It would seem that this species has almost disappeared from even the limited area of distribution in the town referred to by Ross, Annett and Austen. To-day, with one exception, it has not proved possible to find breeding places of this species without going outside the inhabited areas of the town. This apparent modification of the distribution may be compared with what may prove to be a similar modification in the distribution of *A. rhodesiensis* which, formerly not recorded from the town itself, has been found by us in scanty numbers in several widely separated spots.

Meantime, it may be said of *A. funestus* that, however important it may prove to be as a carrier of malaria outside the town, it is quite unimportant in Freetown itself when compared with *A. costalis*.

A. rhodesiensis Theo.

This species was found to occur sparsely over a wide area including a large portion of the town and the surrounding country; fourteen breeding sites were discovered but the number of larvae found at each site was small. The largest number found was fifteen at one spot (Record No. 205), which was on the outskirts of the town near the Public Works Department. The species was also found on the eastern side of the town as far as Kissy and on the south-western as far as Hill Station, where the larvae were found by

officials of the Sanitary Department, in the water lying in hollows in large flat stones. Although the spot map shows this species to have a wide distribution it must be emphasized that it is extremely rare when compared with *A. costalis*. Its breeding sites and the associated species indicate its adaptability; in the five places in which it was encountered within the town boundary it was always associated with *A. costalis*; in two sites outside the town, in Nicol's Brook, with *A. smithii*, in another in the upper part of Granville Brook, with *A. funestus*, and in still another, at Kissy Flats, with *A. costalis* and *A. squamosus*. Considering the difference in the character of the breeding sites of these associated species, it is obvious that *A. rhodesiensis*, which for some reason is not a numerous species here, is one which is capable of adapting itself to a great variety of breeding places. It was found, for example, beside a stream almost at sea level (Pl. V, fig. 1); at an altitude of 800 feet; in streams; in road-side ditches; in rock-pools; and, finally, in a concrete washing tank (Pl. V, fig. 4). It is not possible to explain at present why this species has not previously been recorded from Freetown, although it has been recorded by Evans (1924) from close to it at the Cape Lighthouse Peninsula, and it is known from other parts of Sierra Leone. It may be that owing to its sparseness it has been overlooked in previous surveys, or that it has a definite and short breeding season; again it may be that it usually fails to develop under laboratory conditions, or finally, that it may be a recent addition to the Anopheline fauna of Freetown. We are not in a position to decide which of these alternative explanations is the correct one. It is not even possible to say whether *A. rhodesiensis* is an efficient carrier of malaria here and at present it would be a matter of some difficulty to obtain sufficient adults for the performance of transmission experiments.

A. smithii Theo.

This species was only found outside the town in the upper, hilly portion of Nicol's Brook and the Congo River. Thus it is limited to those parts of these streams which are steep, rocky and shaded by overhanging trees.

A detailed survey of this species was made in the first of the two rivers referred to.

Starting from the point just at the boundary of the town where *A. costalis* ceased to be present, *A. smithii* was suddenly found in large numbers. The amount of overlapping of the breeding places of these two species on this stream was limited to the occurrence of isolated specimens of each species in the area of distribution of the other. The stream was traced up Mount Aureole to its sources on Kortright, at a level of over 1,000 feet. The larvae of *A. smithii* were found in large numbers in pools in the rocky bed of the stream. They were mainly concentrated in two areas of the stream, one near the lower limit of their distribution, above the town boundary, and one near the upper limit not far from the source of the stream. In the intervening length of stream they were found in small numbers only.

The general characters of the breeding places of *A. smithii* are as follows. The breeding places (see Pl. V, fig. 2) are on a rocky bottom which is covered as a rule with dead leaves, often with little or no living vegetation; the water in the pools, though apparently still, was usually found to be in direct communication with the current of the stream. A considerable amount of shade was usually present, but the larvae might be exposed to the sun for certain hours daily. Larvae were, however, also found in the margins of still pools where there was abundant vegetation at the edge of the water. In one case larvae were not seen until a tangled mat of roots and creepers, which lay on the rocky bottom of the pool at the side, was lifted up, when numerous larvae were discovered. On two occasions several larvae were found in small pools sunk in the banks of the stream, the water in them almost hidden from view by a tangle of sticks and creepers.

The extreme localisation of this species, which has not hitherto been recorded elsewhere than at Mount Aureole, is of interest. The only records of it so far as we are aware are those of Theobald, who described the species from several female specimens captured on Mount Aureole by Major Smith, R.A.M.C., and, later, referred a female specimen captured by Captain Grattan, R.A.M.C., at Aureole, to this species. Captain Grattan took male specimens of this species and Theobald made them the type species of the genus *Feltinella*, calling them (*A.*) *Feltinella pallidopalpi*; Christophers (1924), in his catalogue of the Anophelines, makes *A. pallidopalpi* a

synonym of *A. smithii*. From the result of breeding out more than a hundred adult specimens from larvae we are able to confirm this synonymy.

It was found that this species continued breeding throughout the wet season, wherever suitable pools occurred in hilly portions of the streams. Searches made at intervals during the period of investigation continued to yield larvae of all stages and also pupae until the investigation ceased on September 9.

A. marshalli var. *freetownensis* Evans.

The larvae of this species were found on eight occasions in rock-pools in certain streams outside the town, but were never found in the town itself. Three records were from Nicol's Brook, at and above Aureole Bridge, three from streams near Kissy Bridge, one from a stream flowing to Granville Brook at Cline Town, and one from a tributary of the Congo River at Hill Station. The character of the breeding places may be gathered from the fact that in four cases they were associated with the larvae of *A. smithii*, in one with those of *A. costalis*, and in another with those of *A. funestus*. When found alone the larvae were in streams where a certain amount of shade was present. The larvae were found from just above sea-level to 800 feet above sea-level.

A. squamosus Theo.

Larvae of this species were found in one locality only, namely in Kissy Flats, where they were associated with those of *A. costalis*. The site was a shallow unshaded pool in the course of a ditch in laterite, with slowly running water and some vegetation.

A. theileri Edw.

Only four records of this species were obtained. Larvae were found once in association with *A. rhodesiensis* and *A. marshalli* var. *freetownensis*, once with larvae of *A. rhodesiensis*, and on two occasions they were not associated with any other species. Three of the records were, however, from places situated far apart; one being situated well outside the eastern, the others outside the south-western boundary of the town.

A. domicolus Edw.

A single specimen reared from a pupa taken in a road-side ditch at Regent, above Freetown, was referred provisionally to this species.

During the course of this investigation material was examined from various natural, small collections of water, contained in tree holes, axils of leaves, and bamboo, pineapple and banana stems. Numerous samples from such sites were examined and in spite of the fact that larvae of some species of mosquito were present in 179 cases, it was remarkable that in no case were the larvae of Anophelines found in such sites. Owing to the closure of wells in Freetown the findings of Allan (1913), who discovered *A. costalis* in twenty-eight of 418 wells, cannot, fortunately, be repeated.

SUMMARY OF CHIEF POINTS BROUGHT OUT BY THE SURVEY

Eight species of Anopheles were found either in or near Freetown.

A. costalis is still by far the most numerous species in the town itself.

The chief breeding places of *A. costalis* are in and near the courses of the four streams which flow through the town.

During the heavy rains, shallow laterite drains in streets that have no slope are also important sources of larvae. Larvae of this species were always found in situations exposed to a considerable amount of sunlight; they were usually in entirely unshaded places.

A. funestus is found rarely in water courses on the eastern and southern sides of the town; it was taken once in the town itself, this being the first record of its presence within the town boundary since 1901.

A. rhodesiensis was found to be widely distributed in Freetown and the surrounding hills. It was the species found in situations exposed to the sun at Hill Station.

A. smithii. Larvae of this species were very numerous in the pools in the wooded upper part of Nicol's Brook, especially from the middle of May till the end of July. In the height of the rains larvae could only exist in the pools or eddies of the stream sheltered from the force of the current. They were also taken in the Congo River

system just below Hill Station. Considerable tree-shade was usually, but not invariably, present at the breeding places.

A. marshalli var. *freetownensis* occurred outside the western, southern and eastern boundaries of Freetown, but not in the town itself. The larvae were almost always found in streams and usually in shaded situations. They were often associated with those of other species.

Other species found were *A. squamosus*, larvae of which were taken in ditches at Kissy, *A. theileri*, larvae of which we found in streams at Hill Station and to the east and south-west of the town, and *A. domicolus* Edw., taken in a ditch at Regent, behind Freetown.

KEY TO THE FOURTH STAGE LARVAE OF ANOPHELINES OCCURRING IN SIERRA LEONE.

1. Antenna with branched hair on shaft..... *mauritanus**
- Antenna without branched hair on shaft.....2
2. Dorsal plates relatively very large (fig. 4, A)..... *funestus*
- Dorsal plates relatively small and inconspicuous
(fig. 4, B and D).....3
3. Outer clypeal hairs (fig. 1, A) plumose..... *squamosus*
- Outer clypeal hairs simple or slightly branched
(fig. 1, B, C).....4
4. Outer clypeal hair more than three-fourths the
length of the inner (fig. 1, C)..... *smithii*
- Outer clypeal hairs not more than three-fifths the
length of the inner5
5. Thorax without palmate hairs†..... *costalis*
- Thorax with palmate hairs.....6
6. Leaflets of palmate hairs on third to seventh
abdominal segments without filament (fig. 2, B)...*theileri*
- Leaflets of palmate hairs on third to seventh segments
with filament (fig. 2, A, C-G).....7
7. Palmate hairs on fifth abdominal segment very large,
c. 0.2 mm. in diameter (fig. 2, C), sub-median
thoracic hairs with bases enlarged and fused
(fig. 6, D)..... *marshalli* var. *freetownensis*
- Palmate hairs on fifth abdominal segment smaller,
c. 0.15 mm. in diameter (fig. 2, A), sub-median
thoracic hairs with bases well separated (fig. 6, B)... *rhodesiensis*

* The larva of *A. obscurus*, Grünb. probably possesses this character.

† Palmate hairs are here understood to be structures having a short stout base from which arise distally a number of leaflets mostly sub-equal in length.

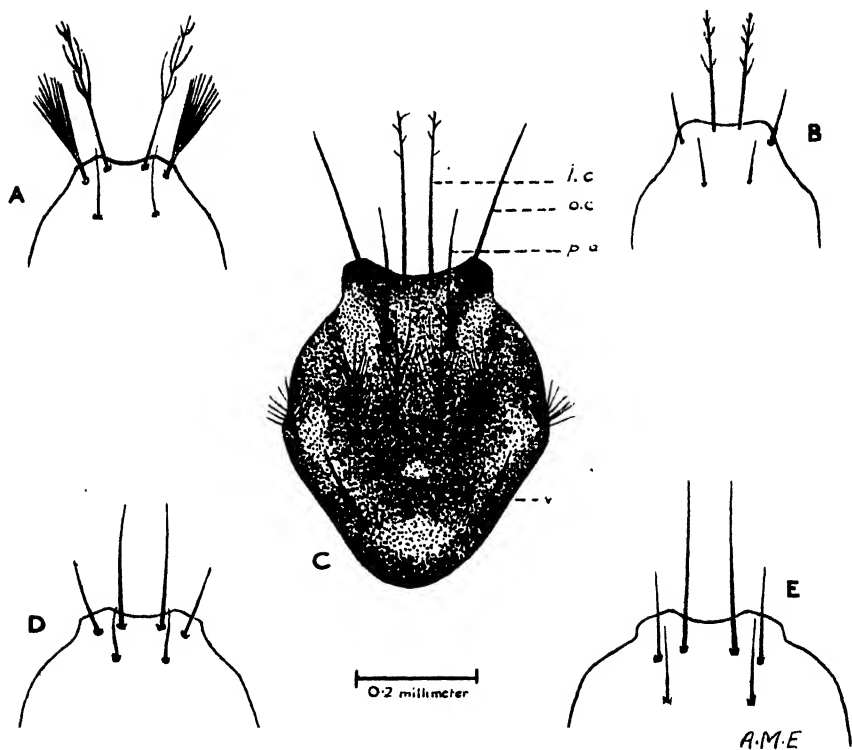


FIG. 1. Clypeal and post-antennal hairs of A.—*A. squamosus*; B.—*A. costalis*; D.—*A. rhodesiensis*; E.—*A. marshalli* var. *freetownensis*; C.—clypeus and vertex of *A. smitbii*; *i.c.*—inner clypeal hair; *o.c.*—outer clypeal; *p.a.*—pre-antennal; *v.*—vertical.

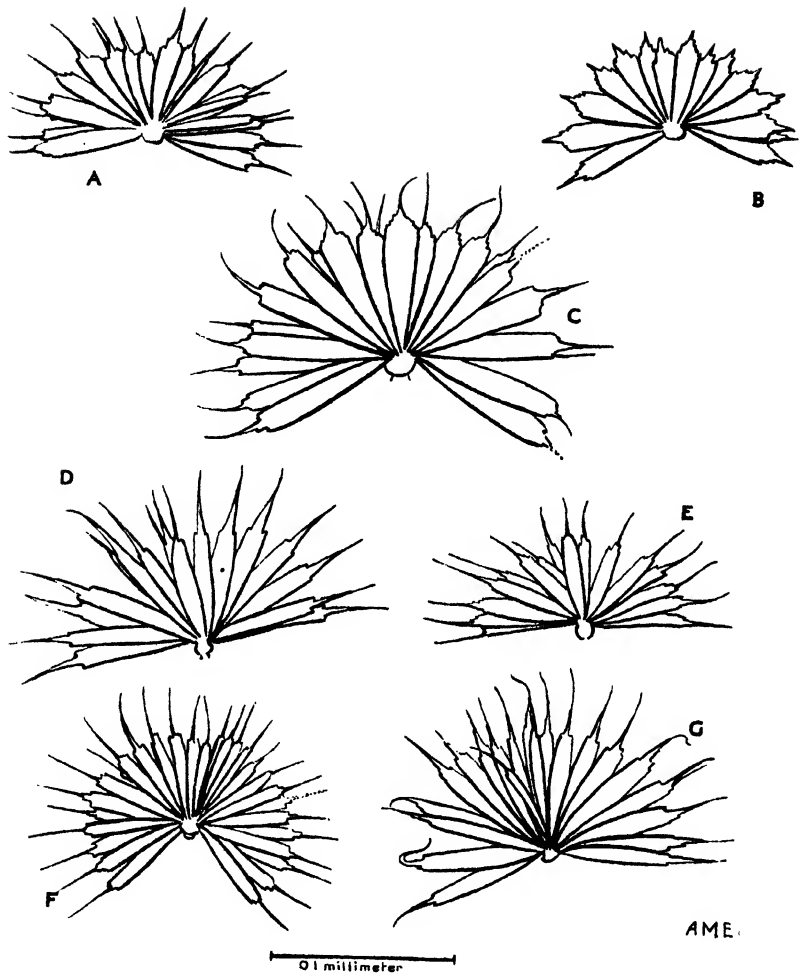


FIG. 2. Palmate hairs of fifth abdominal segment. A.—*A. rhodesiensis*; B.—*A. theileri*; C.—*A. marshalli* var. *freycinetensis*; D.—*A. squamosus*; E.—*A. costalis*; F.—*A. funestus*; G.—*A. smithii*.

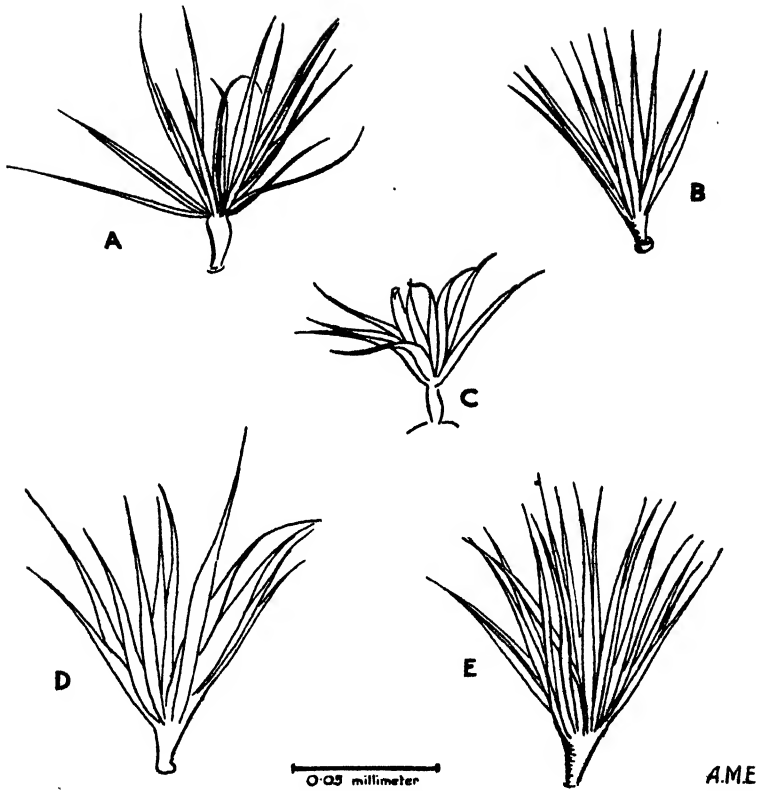


FIG. 3. Palmate hairs of thorax. A.—*A. funestus*; B.—*A. rhodesiensis*; C.—*A. squamosus*; D.—*A. smitbii*; E.—*A. marshalli* var. *freetownensis*.

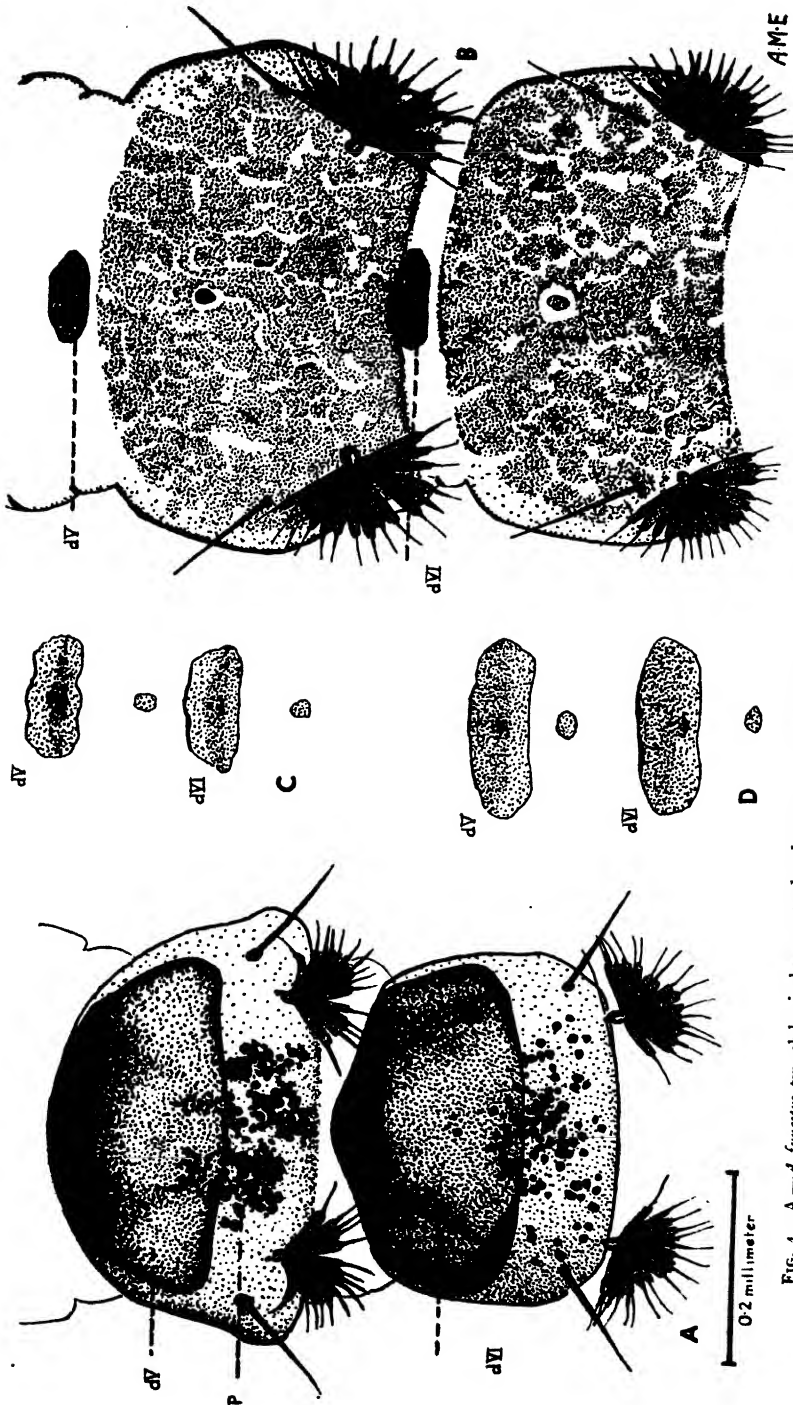


FIG. 4. A.—*A. funetius*, two abdominal segments, dorsal aspect; B.—*A. smithi*; C.—*A. mariballi* var. *freetownensis*, dorsal plates of two abdominal segments; D.—*A. rhodesiensis*; dIV, dVI—dorsal plates of fifth and sixth segments respectively; p—pigment.

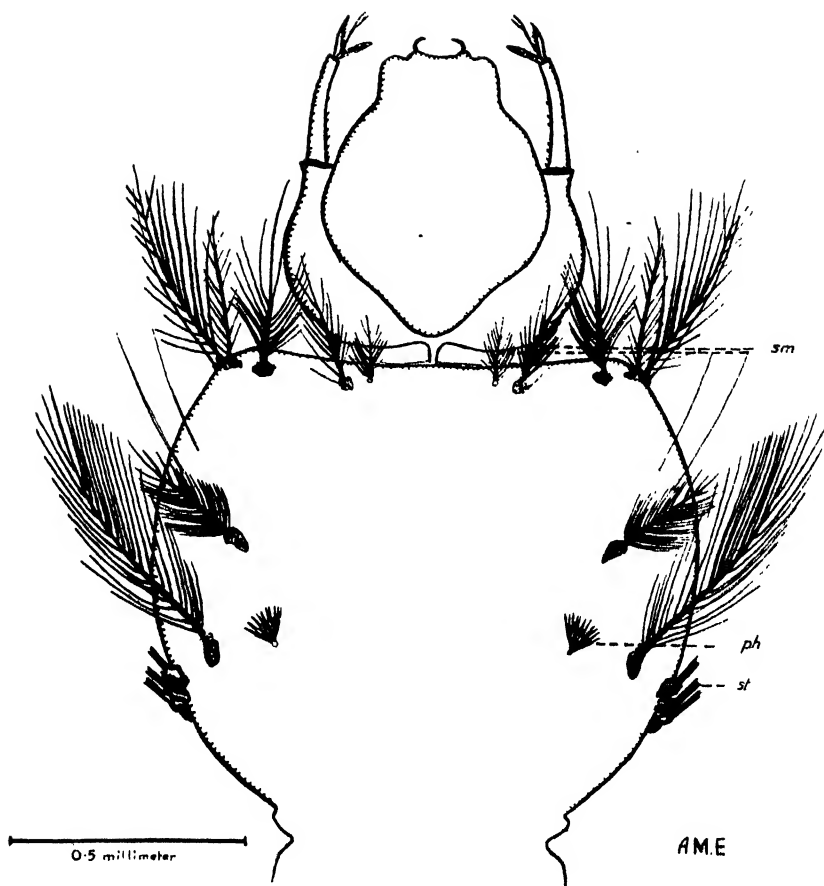


FIG. 5. *A. smitbii* (?), diagram showing position of sub-median thoracic hairs; *p.b.*—palmate hair; *s.m.*—sub-median hair; *st.*—stems of plume hairs.

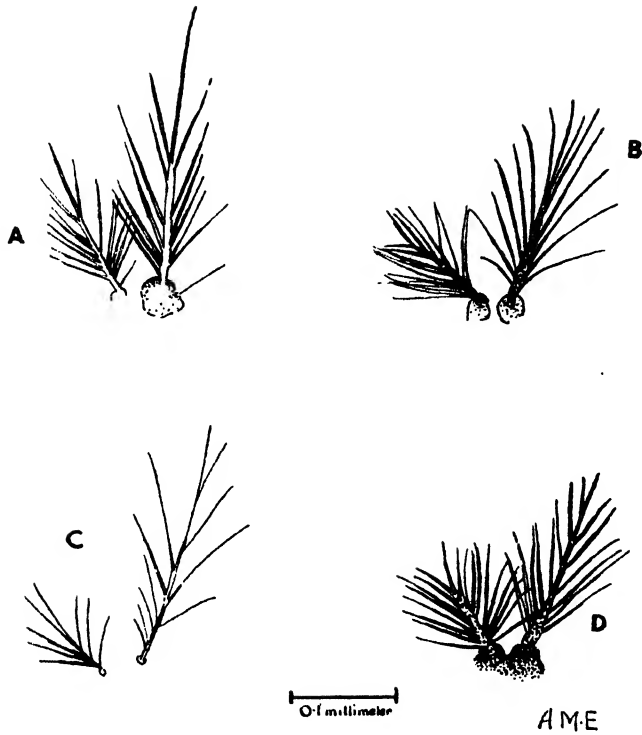


FIG. 6. Sub-median thoracic hairs of A.—*A. smitbii*; B.—*A. rhodesiensis*; C.—*A. costalis*; D.—*A. marshalli* var. *freetownensis*.



FIG. 7. Lateral combs. A.—*A. smitbii*; B.—*A. theileri*; C.—*A. squamosus*; D.—*A. marshalli* var. *freetownensis*; E.—*A. rhodesiensis*.

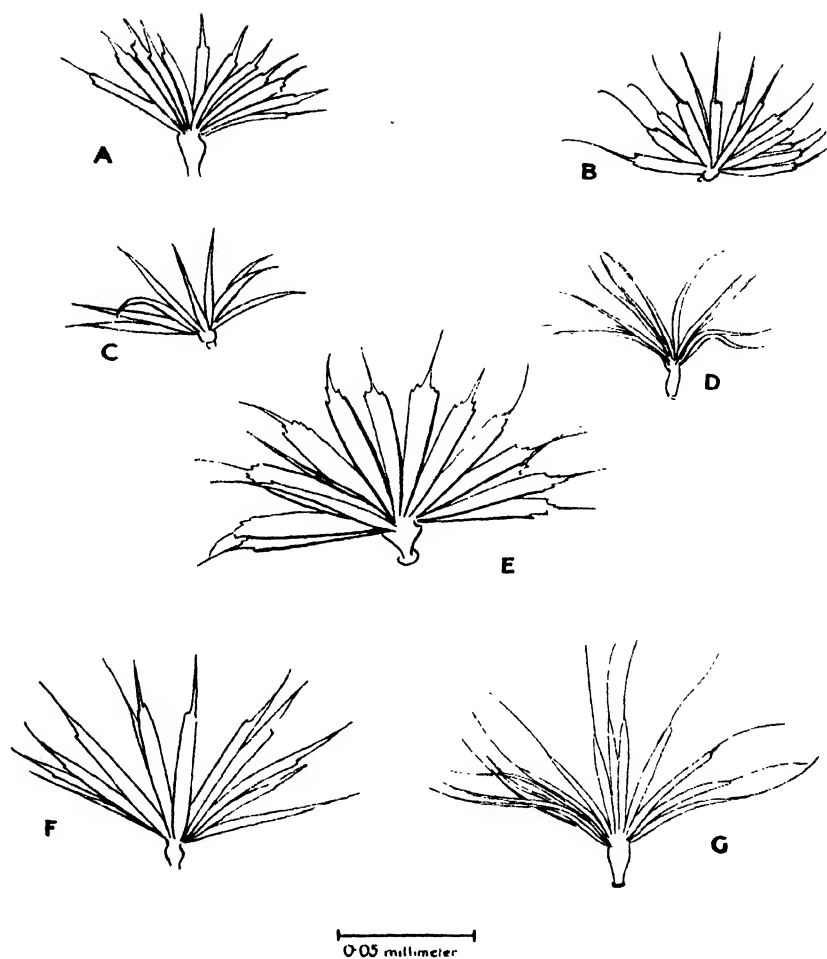


FIG. 8. Palmar hairs of first abdominal segment. A.—*A. theileri*; B.—*A. smitbii*; C.—*A. rhodesiensis*; D.—*A. costalis*; E.—*A. marshalli* var. *freetownensis*; F.—*A. squamosus*; G.—*A. funestus*.

**DESCRIPTIONS OF THE LARVAE OF THREE SPECIES OF
ANOPHELES AND NOTES ON THE DISTINCTIVE CHARACTERS
OF OTHERS**

A. smithii Theo.

Larva. The fourth stage larva is almost invariably dark, appearing black to the naked eye. When examined microscopically the pigment is usually seen to have a bluish green hue. The clypeal hairs are very coarse, the outer are divergent and almost equal in length to the inner. The thorax bears small rudimentary palmate hairs and the lateral combs have the teeth all long and approximately equal in length, except that at the ventral end one or two short teeth occur.

Head. Head capsule unusually heavily chitinised, deep red-brown to black. Antennae with no branched hair on shaft, spines sub-equal, hair divided into two. Inner clypeal hairs (fig. 1, c) long, with delicate branches apically, sometimes extending backwards over the distal third. Outer clypeal hairs as stout as, and only slightly shorter than, inner clypeal, simple, divergent. Pre-antennal more than half as long as inner clypeal, stout, finely plumose; post-antennal branched; occipital simple or bifurcated. Mental plate relatively very narrow. *Thorax.* Plume hairs well developed, sub-median hairs (figs. 5 and 6, A) rather long and slender, the plates at their bases well separated. Palmate hairs (fig. 3, D) with about eleven long slender leaflets. *Abdomen.* Palmate hairs of first abdominal segment (fig. 8, B) small, but leaflets well developed with marked shoulder and filament. Palmate hairs of segments three to seven relatively large, that of fifth segment (fig. 2, G) with longest leaflets about 0.09 mm. and the greatest width about one-ninth of this. Shoulder with one, two or (rarely) three serrations, filament originating rather gradually, length (taken from distal serration to tip) about two-sevenths of the total length of the leaflet and filament. Lateral combs with about eleven sub-equal teeth, markedly serrated. Dorsal plates very small (fig. 4, B). One or two short teeth occur towards the ventral aspect.

A. marshalli var. *freetownensis* Evans.

Larva, Fourth Stage. In life the larva is usually dark grey with blackish pigment; the dorsal plates are small, and palmate hairs

are present on the thorax and first seven abdominal segments ; those of the third to seventh segments are relatively very large.

Head. Antennae with no branched hair on shaft ; terminal hair divided into two or three. Inner clypeal hairs (fig. 1, E) generally simple, occasionally with fine lateral secondary hairs towards the extremity. Outer clypeal hairs simple, slightly less than one-half the length of the inner ; pre-antennal, simple, about equal to the outer anterior clypeal ; post-antennal, branched, occipital simple. *Thorax.* Plume hairs well developed. Sub-median hairs (fig. 6, D) arising from chitinous plates, those of each pair being fused. Palmate hairs (fig. 3, E) large with about eighteen long narrow leaflets. *Abdomen.* Palmate hairs of first segment comparatively large (fig. 8, E) leaflets with well-developed shoulder and filament ; palmate hairs of segments three to seven unusually large, that of the fifth segment (fig. 3, E) with leaflets large (total length c. 0.1 mm., width from one-eighth to one-ninth of this), the shoulder much serrated and the filament long and slender, about two-sevenths of the total length of the leaflet. Dorsal plates of normal size (fig. 4, C). Lateral combs (fig. 7, D) with a rather great disparity between the long and short teeth, all the teeth finely serrated.

The larva of this species differs markedly from that of *A. marshalli* Theo., in which the first and second abdominal segments as well as the thorax are devoid of rudimentary palmate hairs (Macfie and Ingram, 1917).

A. costalis Theo.

The larva of this species has been fully described by Hill and Haydon (1907). It can usually be distinguished from the other species by the colour, the pigmented portions being pale brown or, sometimes, green, and the lateral part of the thorax opaque creamy-white. The small size of the abdominal palmate hairs (fig. 2, E) is a constant character.

A. funestus Giles.

As Edwards (1922), and subsequent authors, have pointed out, this species and its allies differ from other *Anopheles* in the larval stage by the relatively enormous size of the dorsal plates (fig. 4, A).

A. rhodesiensis Theo.

The larva of this species has been described by Christophers and Chand (1916).

Specimens found in the neighbourhood of Freetown are usually very dark, appearing dark grey or blackish to the naked eye, and have paired darker quadrangular areas on the abdominal segments. They may be distinguished from the larvae of *A. costalis* by the presence of small palmate hairs on the thorax (in the living larva these can be seen best by using reflected light), and by the unbranched inner clypeal hairs. The larvae are more difficult to distinguish from those of *A. marshalli* var. *freetownensis*, but the well-separated bases of the sub-median thoracic hairs can readily be seen and the large size of the abdominal palmate hairs in var. *freetownensis* is a striking character.

A. squamosus Theo.

Hill and Haydon (1907) have described the larva of this species in detail. The dendriform, outer clypeal hairs (fig. 1, A) distinguish it at once from the other species which have no branched hair on the shaft of the antenna. *A. pharoensis*, however, shares this character, and Edwards (1912), in his key to the larvae of the African Anophelines, does not distinguish between the two species.

A. theileri Edw.

There seems to be no difficulty in separating the larvae of this species from those of others likely to be met with in Sierra Leone. In addition to possessing the characters given in the key, the larva is unusual in having the smaller teeth of the lateral combs (fig. 7, B) extremely short.

A. obscurus Grünb.

Christophers (1924) has recently separated the African form of *A. umbrosus* Theo. under the name *A. obscurus* Grünb. From the close resemblance between the adults of the two species it seems safe to assume that the larvae of *obscurus* will have the same general characters as those of *umbrosus*.

Anopheles nili Theo.

The accompanying illustrations (figs. 9 and 10) show the characteristic features of a larva found in the Moa River, at Daru, by the senior author in 1924.

The larva was one of several taken from the edge of the river, many of which hatched out, giving rise to two species only, namely, *A. costalis* and *A. nili*. There is, therefore, very little doubt that this larva is that of *A. nili*. A similar but slightly imperfect larva was found in a swamp near Daru. The following is a description of the larva.

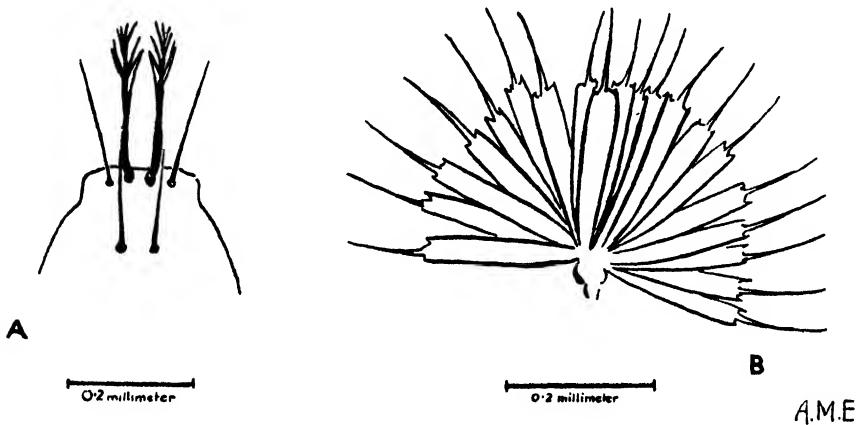


FIG. 9. *Anopheles* sp ? *nili*. A.—clypeal and post-antennal hairs; B.—palmate hair of thorax.

Head. Antennae with no branched hair on shaft. Inner clypeal hairs (fig. 9, A) very thick, widest a short distance beyond the base, distal third with several rather long coarse branches. Outer clypeal hairs slender, simple. Pre-antennal hairs reaching to the edge of the clypeus, simple. *Thorax.* Palmate hairs (fig 9, B) situated at the posterior angles, very large, *c.* 0.14 mm. in diameter, the leaflets with well-developed shoulder and filament. *Abdomen.* Palmate hairs (fig. 10, B) relatively large, the leaflets with filament and rounded, serrated shoulder, markedly incised between serrations. (It should be noted that the palmate hairs in figs. 9 and 10 are drawn to different scales.) Dorsal plates (fig. 10, A) wide, resembling in shape the thicker basal portions of the dorsal plates of *A. funestus*. Lateral combs with long and short teeth.

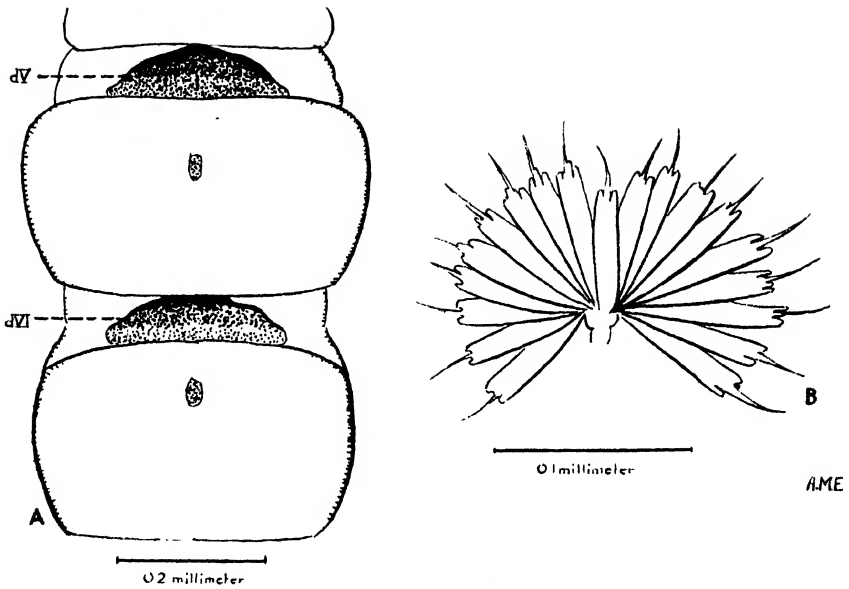


FIG. 10. *Anopheles* sp. ? *nil*i. A.—outline of two segments of abdomen; B.—palmate hair of fifth abdominal segment; *dV*, *dVI*.—dorsal plates of fifth and sixth segments respectively.

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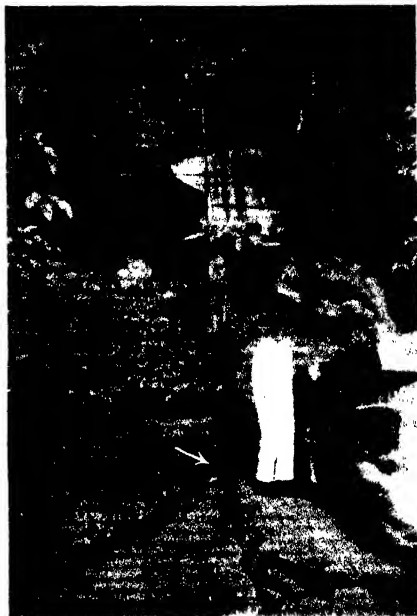
EXPLANATION OF PLATE V

Breeding places of Anopheles.

- FIG. 1. Breeding place of *A. rhodesiensis* in the town. The white arrow is pointing to a small pool from which larvae were taken.
- FIG. 2. Breeding place of *A. smithii* above Aureole Bridge. The small rock-pool indicated by the arrow contained larvae of this species.
- FIG. 3. Breeding place of *A. funestus* just outside the town. The lower barb of the arrow is pointing to the floating weeds among which the larvae were found.
- FIG. 4. Breeding place of *A. rhodesiensis* and *A. costalis* in the town.



1.



2.



3.



4.

Photographs by B. Blacklock

C. Tinling & Co., Ltd., Imp.

THE CULTIVATION OF *ENDAMOEBA* *RANARUM**

BY
HARVEY P. BARRET
AND
NANNIE M. SMITH

(Received for publication 10 November, 1925)

When the present authors began the work on the cultivation of the intestinal amoebae in the cold-blooded vertebrates in 1922, it was hoped that the work would not only be of interest in itself, but might throw some light on the cultivation of the forms from man. At that time the only apparent successful cultivation of a species of *Endamoeba* was the work of Cutler, 1918, on *E. histolytica*, and his work was not accepted by many protozoologists. We succeeded first in cultivating an *Endamoeba* from the turtle (Barret and Smith, 1924). In a footnote to the same paper we noted that we had carried one strain of *E. ranarum* for more than two months. We considered the cultivation of *E. ranarum* to be of especial interest because of the great similarity in structure between this form and *E. histolytica* of man. Since our work began, Boeck and Drbohlav (1925) have undoubtedly succeeded in cultivating the parasite of amoebic dysentery, and Chiang (1925) has cultivated a very similar form (*E. histolytica* var. *murina*) from the rat. Drbohlav (1925, a, b, and c) also reports the cultivation of *E. gingivalis*, *E. coli* and *E. aulastomi*. The object of the present paper is to give the details of the successful cultivation of *E. ranarum* from tadpoles.

* From the Laboratory of Dr. H. P. Barret, Charlotte, N.C., and the Department of Hygiene and Bacteriology, University of Chicago.

CULTURE MEDIUM USED

The culture medium used is the same as that described in an earlier paper in the cultivation of *Blastocystis* (Barret, 1921). It consists of one part of inactivated human serum and nine parts of 0.5 per cent. salt solution.

The H-ion concentration of the medium varies from 7.6 to 7.8 in its natural state. Experimentally it was found that the amoebae would grow in media, ranging from pH 5 to pH 10, but that the most favourable range is between 7 and 8.

PROCEDURE

The lower end of the large intestine of a tadpole is removed, placed on a sterile slide and covered with a small quantity of the culture medium. The contents of the intestine are expressed, and after being mixed with the culture medium, are drawn up in a capillary pipette and placed in two or more tubes containing the medium to a depth of about 50 mm. The material is always placed in the bottom of the tubes as growth takes place only in that portion. The tubes are then placed in the icebox and are allowed to remain undisturbed for from ten days to two weeks, and in some instances longer, after which the sediment is examined for amoebae. One of the mistakes made in the initial attempts to culture the amoebae was an examination of the sediment after too short an incubation period, a fact which may account, in part at least, for the low percentage of positive cultures in our earlier work. When a positive culture is found, transplants are made into several tubes every week or two, depending on how rapidly the amoebae grow. In our routine procedure transplants were never made under one week and some strains did better if left undisturbed for two or even three weeks.

Many obstacles were encountered, which added greatly to the labour of the work. At first, the contents of the lower end of the large intestine were only sub-cultured if they were microscopically found positive for amoebae. Experience showed that this procedure was detrimental to the amoebae, because of drying, etc. Instead, the contents of each intestine were treated exactly as if they contained amoebae and microscopical examination was deferred until the appropriate time for sub-culturing. In the second place, infected frogs and tadpoles are extremely scarce. Some observers have

found as high as 5 per cent. of certain species of frogs infected, while others place the figure at 1 per cent. Owing to this, as well as to the fact that tadpoles are handled in large quantities more easily than frogs, the present workers used tadpoles exclusively. Out of five hundred tadpoles examined, we obtained only six positive cultures, or a little over 1 per cent. infection. In the third place, there were many other organisms in nearly every animal examined which, because of their much more rapid growth, inhibited the multiplication of the amoebae. *Blastocystis* and intestinal flagellates of different kinds were the most troublesome contaminants. In fact, if a culture was contaminated with *Blastocystis* it could neither be freed from them nor successfully transplanted many times thereafter. On the other hand, one of us (Smith) has met with a certain amount of success in freeing cultures of flagellates by subjecting them to varying dilutions of mercurochrome for different lengths of time. The most favourable dilution of mercurochrome was 1-2000; the most favourable time of exposure was one hour.

The writers have been able to carry three strains of *E. ranarum*, obtained from tadpoles 220, 222, and 226 respectively, continuously, through numerous sub-cultures, for more than eight months. Furthermore, these strains at their last transfer showed no evidences of dying, and hence, can apparently be kept going indefinitely.

Cysts were present in practically all cultures after a week's growth, their numbers probably depending on the age of the culture. There seems to be, however, no tendency for the active amoebae to disappear entirely in the very old cultures and their places to be taken by cysts. When a culture died, the cysts and amoebae soon disappeared. In this respect, cultures of *E. ranarum* differ from cultures of free-living amoebae, for in the latter, cultures are viable for months owing to the presence of the cysts alone. As stated above, we have been able to obtain cultures that are free of other protozoa; but all cultures are contaminated with the associated bacteria. From findings in some of the cultures, we believe there is more than one species of amoebae living in the frog. We hope to take up this question later.

A description of the morphology of the amoebae in our cultures is given in the paper by Taliaferro and Fisher, immediately following this one.

CONCLUSION

E. ranarum has been successfully cultivated on a simple medium, and strains have been carried through successive transplants over a period of more than eight months.

LITERATURE CITED

The literature cited in the present paper is given at the end of the paper by Taliaferro and Fisher, which follows.

THE MORPHOLOGY OF MOTILE AND ENCYSTED *ENDAMOEBA RANARUM* IN CULTURE

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AND
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(*Received for publication 10 November, 1925*)

PLATE VI

All of the amoebae used in this study were obtained from cultures started and furnished us by Barret and Smith. These investigators originally started their cultures from amoebae found in tadpoles and a detailed description of their technique will be found in their paper, immediately preceding this one. The present paper indicates beyond reasonable doubt that these authors have successfully cultivated an *Endamoeba* which, in culture, goes through the usual stages observed in the body, viz., active amoebae, precystic amoebae and cysts, and furthermore, that the specimens in all stages of development are identical with the description given by various authors of *Endamoeba ranarum* Grassi, 1879. In our previous study (Taliaferro and Holmes, 1924) of the morphology of an entozoic amoeba from the turtle, which Barret and Smith had cultivated, we deemed it necessary to study the forms from both the turtle and cultures. In the present case, however, where the form with which we are dealing has been studied by many investigators who have given excellent descriptions of it, it seemed necessary to consider only the amoebae from the cultures.

At the outset we wish to emphasize that a morphological study is always essential before an author is justified in concluding that he has actually cultivated an entozoic species. In fact, omissions of

this kind have been the basis of much confusion in the earlier attempts to cultivate entozoic amoebae. Thus, free-living contaminants have been mistaken for the entozoic species which were supposed to have been cultivated.

Since a review of the earlier work may be found in Dobell (1909) and much of the later work in Nöller (1922), no attempt will be made to review the various papers on the morphology of *E. ranarum*. Suffice it to say that Dobell has given an accurate description of the organism—a description which agrees in all major details with that given in the present paper. Moreover, not only can we compare the present cultural forms with the description by Dobell, but we can also make use of the resemblance between *E. ranarum* and *E. histolytica*. This will be most helpful, since the structure of *E. histolytica* has been so widely studied and is so well known. Dobell, in a footnote to the paper cited, calls attention to the extraordinary 'resemblance' between Hartmann's figures of *E. tetragena* (= *E. histolytica*) and isolated stages in *E. ranarum*. This remarkable resemblance between *E. histolytica* and the parasite of the frog has been noted by several subsequent workers. It even led Alexeieff (1914) to suggest that the harmless commensal of the frog, when introduced accidentally into man, might become the parasite of amoebic dysentery. The infection experiments of Dobell (1918) invalidate this conclusion, however, and indicate that the two species are distinct. With regard to the morphological similarity of the two, Dobell (1918) states, 'The active amoebae can usually be readily distinguished from one another by the inclusions (food bodies) in their protoplasm, but not by their own nuclear and cytoplasmic structure; but the precystic amoebae, devoid of all food bodies, and the cysts, at every stage of development, are so closely alike that preparations of the one could be used as demonstrations of the other.'

MATERIAL AND METHODS

As previously stated, all of our material was supplied us in culture by Barret and Smith. These cultures were subcultured on Barret and Smith's medium in this laboratory for several months. In the previous work of Taliaferro and Holmes (1924) on *Endamoeba barreti*

from the turtle, it was found that rabbit serum or Loeffler's dehydrated beef serum could be substituted for human serum in the original Barret medium. In our rather limited experience with *E. ranarum*, on the other hand, we have not obtained anything like as satisfactory results with rabbit or pig serum, Loeffler's dehydrated beef serum or human ascitic fluid, as we did with human serum.

All observations of living specimens were carried out at room temperature with material mounted under cover glasses sealed with vaseline. All of the prepared slides were fixed in Schaudinn's alcohol-sublimated mixture with 2 per cent. acetic acid. They were stained with Delafield's or Heidenhain's iron-haematoxylin with and without counter stains. No fixative was used to get the amoebae to adhere to the slide. As a consequence, the larger cysts tended to wash off the slides during the process of staining and dehydration (see differences in measurements of living and prepared cysts).

We have made a very careful study of the size of the present form in all three stages of its development, viz., active and precystic amoebae and cysts. In all cases the forms were drawn with a camera lucida, generally at a magnification of $\times 3,000$, and the drawings measured. Owing to the characteristic circular shape of the precystic amoebae and the cysts, their size is given as their diameter in microns, but, since the active amoebae are generally very irregular in outline, their measure of size is given as the diameter of a circle having approximately the same area as the amoeba. (This method is similar to that used by Taliaferro and Holmes on *E. barreti*.)

With regard to size, active amoebae were measured from prepared slides; (1) from a culture containing only active forms, i.e., containing no cysts; (2) from a culture in which cysts had just begun to appear, and (3) from a month-old culture in which there was a large number of cysts. Their range in size varied as follows:—

| No. measured | Range | Average |
|--------------|--------------------------|------------|
| (1) 60 | 18·3 μ to 38·0 μ | 26·3 μ |
| (2) 53 | 12·0 μ to 38·5 μ | 23·1 μ |
| (3) 45 | 13·2 μ to 26·0 μ | 19·0 μ |

The progressive decrease in average size which is brought out by these measurements is just what would be expected, since parasitic amoebae habitually grow smaller in preparation for encystment. In

passing, we may note that the size of these forms is within the range of size recorded for *E. ranarum* in the literature. Furthermore, the size of the large active amoebae (18.3μ to 38.0μ , average 26.3μ) is quite similar to the size of *E. histolytica*. Dobell (1919) gives the usual range of *E. histolytica* as 20μ to 30μ , and the extreme range as 18μ to 40μ .

The range in size of cysts studied in iodine was 9.6μ to 20.6μ , with an average of 14.8μ . Later, 105 cysts from the same culture, but drawn from prepared slides, showed a range of 6.3μ to 14.5μ , with an average of 10μ . In view of Dobell and Jepp's (1918) study of the size of *E. histolytica*, we might have expected a slight decrease in diameter (about 10 per cent.), but nothing like the one observed. We believe that the discrepancy is due simply to the fact that the smaller cysts adhere to the slide better than the larger ones. Indeed, this is borne out by the fact that in destaining the slides, one can actually see the large cysts being washed off. Therefore, we are probably justified in giving the range of size of the cysts in our cultures as from about 6μ to 20μ . The great similarity of these measurements to those of both *E. ranarum* and *E. histolytica*, is apparent when it is recalled that the diameter of cysts of *E. ranarum* is given as 10μ to 16μ by Nöller (1922) and of *E. histolytica* as 5μ to 20μ , by Dobell (1919).

The appearance of the active amoebae in the cultures depends largely on the food available. In the first cultures which contained flagellates, the endoplasm was generally so loaded with these organisms as to obscure the nucleus. In later cultures which were free of flagellates the motile amoeba appeared much like that shown in fig. 1. Specimens, for some time after being placed on a slide, assumed an elongated shape and progressed by means of lobose pseudopodia. These were extruded as masses of clear ectoplasm into which, later, the endoplasm flowed, and were formed from alternate sides of the anterior portion of the animal (fig. 1). When the slide began to dry up, the amoebae generally assumed a more spherical shape and extruded—almost explosively—clear hyaline pseudopodia without any evidence of active progression. In this condition they were almost identical in appearance with *E. histolytica* as seen in ordinary mounts of fresh faeces. The nucleus could be generally seen as a ring of dense material in which was embedded a

number of bright refractile granules (fig. 1). The karyosome was rarely visible in the living specimen although it was visible in the specimen from which fig. 1 was drawn.

The general appearance of the active amoebae in stained preparations is shown in fig. 6, and the nuclei of two other specimens in figs. 7 and 8. The endoplasm of the specimen in fig. 6 was crowded with food vacuoles containing flagellates. The structure of the nucleus is interesting because it possesses all of the distinguishing characteristics of *E. histolytica*, such as the delicate layer of chromatin around the periphery, the small centrally-placed karyosome which is shown in figs. 7 and 8, and the linin network between the karyosome and periphery which is devoid of chromatin. In deeply-stained specimens, the karyosome cannot be seen (fig. 6)—most probably owing to its being obscured by the overstained linin network.

The large active amoebae frequently have more than one nucleus. In the sixty amoebae drawn to give the measurements discussed in a previous paragraph, six possessed two nuclei and one possessed four.

At no stage do any of the specimens ever show any trace of a contractile vacuole.

In cultures at the height of their growth there is little or no tendency for the amoebae to encyst, but as the cultures grow older the amoebae become smaller and more sluggish. In time these forms lose all food inclusions and become typical precystic amoebae. Fig. 9 shows a specimen which is probably intermediate between the large active form and the precystic form, whereas fig. 10 shows a typical precystic amoeba. These are even more like *E. histolytica* than the active forms. Their nuclei, as shown in fig. 10, are in every respect identical with that of *E. histolytica*, and in our experience, both the karyosome and achromatic capsule are more clearly seen than in the motile forms.

When the precystic forms encyst they are again identical with the cysts of *E. histolytica*. Figs. 2, 3 and 4 show their general appearance when alive. Quite frequently one or two nuclei can be made out in the living cysts, but it is rare to see all four, and sometimes none are visible. One nucleus is barely discernible in figs. 2 and 3, respectively. The chromatoid bodies appear rodlike and the glycogen masses as dull inclusions. The nuclei can be easily counted when the cysts are observed in iodine or stained with iron-

haematoxylin. Fig. 11 shows a cyst with one nucleus which is probably in an early stage of division, and figs. 12, 13 and 14 show mature quadrinucleate cysts. Each of these contain chromatoid bodies so diagrammatically like *E. histolytica* as to need no further description. Of the several hundred cysts examined from culture, none contained more than four nuclei, a condition once more similar to *E. histolytica*, for although there is some evidence that *E. histolytica* occasionally forms a supernucleate cyst with eight nuclei, the occurrence must be extremely uncommon. We have never encountered in our cultures any cysts suggesting the figures of Mercier and Mathis (1918), in which they depict their so-called "schizogonic" cysts.

SUMMARY

1. A detailed description is given of the amoebae from cultures originally isolated, by Barret and Smith, from tadpoles. The structure of these amoebae agrees in minutest detail with the descriptions by other authors of *Endamoeba ranarum* from the frog.
2. In culture, *E. ranarum* goes through the typical development of a parasitic amoeba, eventually forming cysts.
3. In agreement with all recent investigators, the present investigation emphasizes the similarity in structure of *E. ranarum* and *E. histolytica*.

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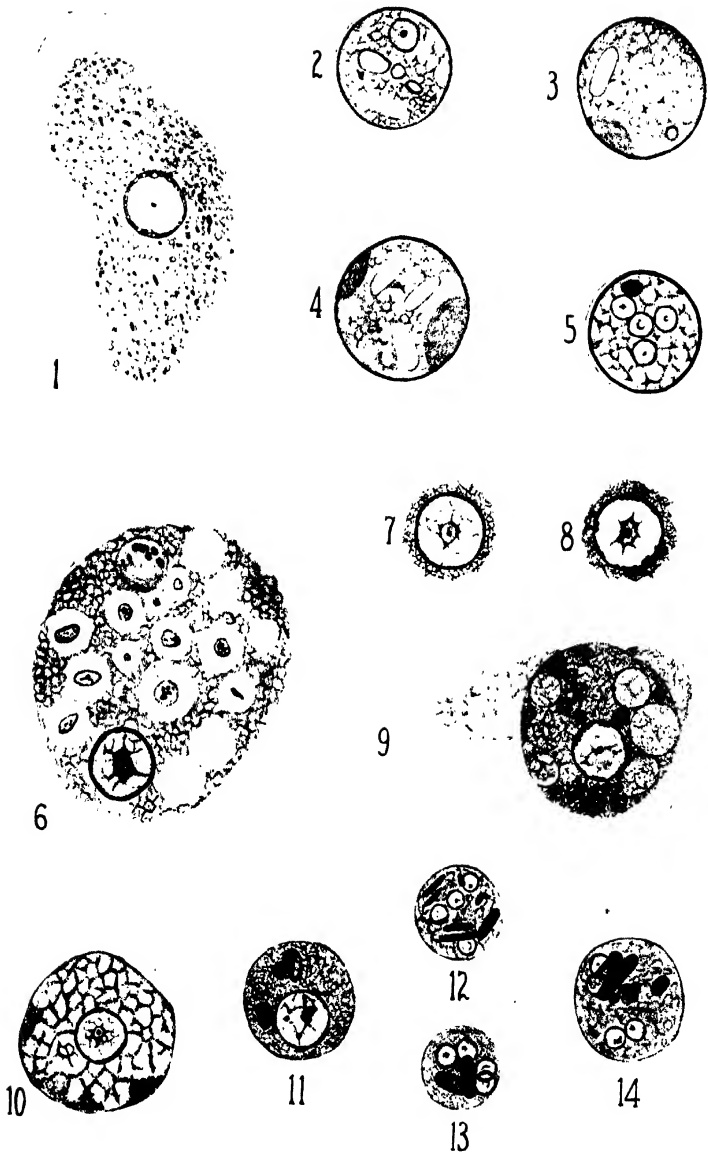
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EXPLANATION OF PLATE VI

All figures are of *Endamoeba ranarum* from cultures in Barret's medium, reproduced at a magnification of $\times 1400$. All drawings were made from camera lucida sketches, although that of the living motile amoeba (fig. 1) was necessarily largely a free-hand drawing. Stained specimens (figs. 6-14) were all fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin without counter stain.

- Fig. 1. Active amoeboid form as seen in living condition from a culture which had been freed of all other species of protozoa. The endoplasm contains a number of rod-like bacteria. Note that the karyosome of the nucleus is visible, although this is an exceptional occurrence in the living organisms.
- Figs. 2, 3 and 4. Cysts from culture as seen in the living condition. Note that each cyst contains chromatoid bodies; that figs. 3 and 4 contain one and two glycogen masses, respectively; and that in figs. 2 and 3, a nucleus is visible.
- Fig. 5. A cyst in iodine from the same culture as the one shown in fig. 2. Note the four nuclei. No chromatoids are visible but there is a rather lightly-stained glycogen mass indicated near the top of the cyst.
- Fig. 6. Active amoeboid form fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin. This form came from a culture which contained intestinal flagellates. The food vacuoles contain débris from the digestion of these flagellates. A definite karyosome is not seen in this specimen.
- Figs. 7 and 8. Nuclei of active amoeboid forms fixed and stained in the same manner as the specimen shown in fig. 6. A karyosome is visible in each.
- Fig. 9. An amoeboid form (fixed and stained as noted) which is probably intermediate between the large active amoebae and the precystic forms.
- Fig. 10. A typical precystic amoeba. (Fixed and stained as noted.)
- Fig. 11. A uninucleate cyst. Four chromatoid bodies are present and the nucleus is probably in a very early anaphase. (Fixed and stained as noted.)
- Figs. 13 and 14. Mature quadrinucleate cysts, all of which contain chromatoid bodies. (Fixed and stained as noted.)



NOTES ON FREETOWN MOSQUITOS, WITH DESCRIPTIONS OF NEW AND LITTLE-KNOWN SPECIES

BY

A. M. EVANS

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PLATE VII

The material dealt with in this paper was collected in the course of a recent survey of the mosquitos of Freetown by Professor D. B. Blacklock and the writer. During the survey very valuable assistance was rendered by Dr. H. O'Hara May, Deputy Director of Sanitary Services, and by the officials of the Sanitary Department, who submitted to us for identification about three hundred consignments of larvae, many of them from tree-holes in Freetown and at Hill Station. I am also indebted to Dr. M. G. Blacklock, Dr. R. M. Gordon and Dr. G. MacDonald, for specimens of larval and adult mosquitos.

The following non-anopheline mosquitos are recorded :—

- | | |
|--|--|
| <i>Culex albiventris</i> Edw. | 1. (<i>Aedimorphus</i>) <i>simulans</i> Newst, and |
| <i>C. annularis</i> Theo. | Carter |
| <i>C. decens</i> Theo. | 1. (<i>Aedimorphus</i>) <i>tarsalis</i> Newst. |
| <i>C. decens</i> var. <i>modiosus</i> Theo | |
| <i>C. duttoni</i> Theo. | <i>Uranotaenia balfouri</i> Theo. |
| <i>C. grabhami</i> Theo. | <i>U. conalli</i> Edw. |
| <i>C. horridus</i> Edw. | <i>U. fusca</i> Theo. |
| <i>C. (Culicomyia) nebulosa</i> Th. | <i>U. nigripes</i> Theo. |
| <i>C. (Culicomyia) cinereus</i> Theo | <i>U. ornata</i> Theo. |
| <i>Lutzia tigris</i> var. <i>fusca</i> Theo. | <i>Hodgesia sanguinis</i> Theo. |
| <i>Aedes (Stegomyia) africana</i> Theo. | <i>Eualbia mediolineata</i> Theo |
| <i>A. (Stegomyia) argenteus</i> Poiret | <i>Harpagomyia trichorostis</i> Theo |
| <i>A. (Stegomyia) blacklocki</i> Evans | <i>Megarhinus brevipalpis</i> Theo. |
| <i>A. (Stegomyia) fraseri</i> Edw. | <i>M. aeneus</i> n.sp. |
| <i>A. (Stegomyia) luteocephala</i> Newst. | <i>M. ? phytophagus</i> Theo. |
| <i>A. (Stegomyia) simpsoni</i> Theo. | <i>Lictmopodites chrysogaster</i> Graham |
| <i>A. (Stegomyia) vittata</i> Bigot | <i>E. chrysogaster</i> var. <i>semisimplipes</i> |
| <i>A. (Finlaya) longipalpis</i> Grunb. | Edw. |
| <i>A. (Aedimorphus) albocephalus</i> Theo. | <i>E. dracaenae</i> Edw. |
| <i>A. (Aedimorphus) apicoannulata</i> Edw. | <i>E. mornatus</i> Newst. |
| <i>A. (Aedimorphus) domesticus</i> Theo. | <i>E. leucopus</i> Graham |
| <i>A. (Aedimorphus) occidentalis</i> n.sp. | <i>E. oedipodius</i> Graham |

The following table shows the results obtained by the identification of Culicine mosquitos from a considerable number of natural sources, including 156 records from rot-holes in living trees. The species of *Megarhinus* and *Eretmopodites* and certain species which occurred in small numbers are not recorded in the table. Many larvae were found in rock-pools in the beds of streams, but as these situations were examined chiefly for the presence of *Anopheles*, the Culicine larvae were in most cases not kept for identification.

TABLE I.

| Situations in which larvae were found | Number of consignments of larvae determined | | | | | | | | | | | |
|---|---|------------------------------|------------------------|-----------------------------|----------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-----------------------------|--------------------|
| | <i>A. (A.) epicoenulatus</i> | <i>A. (S.) luteocephalus</i> | <i>A. (S.) fraseri</i> | <i>A. (A.) occidentalis</i> | <i>A. (F.) longipalpis</i> | <i>A. (A.) simulans</i> | <i>A. (S.) simpsoni</i> | <i>A. (S.) argenteus</i> | <i>C. (C.) nebulosa</i> | <i>A. (S.) vittatus</i> | <i>A. (A.) abbocephalus</i> | <i>U. nigripes</i> |
| Rot-holes in living trees (various) | 43 | 10 | 3 | 2 | 6 | 10 | 5 | 29 | 19 | ... | ... | ... |
| Rot-holes in living mango trees... | 8 | 5 | 4 | 2 | ... | 6 | 1 | 8 | 5 | ... | ... | ... |
| Rot-holes in living pawpaw trees | 2 | 4 | ... | ... | ... | ... | 1 | 8 | 11 | ... | ... | ... |
| Rot-holes in living cotton trees... | 2 | 1 | ... | 2 | ... | ... | ... | 6 | 1 | ... | ... | ... |
| Pool formed by roots of cotton trees | ... | ... | ... | 1 | ... | 1 | ... | 1 | ... | ... | ... | ... |
| Dracaenas | 1 | ... | ... | ... | ... | 1 | 12 | 1 | ... | ... | ... | ... |
| Axils of leaves of liliaceous plants | ... | ... | ... | ... | ... | ... | 5 | 2 | ... | ... | ... | ... |
| Dead stumps of banana plants ... | 3 | ... | ... | ... | ... | ... | 3 | 1 | ... | ... | ... | ... |
| Cut stems of bamboos | 1 | 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Pineapple plants | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Hollows in flat stones | ... | ... | ... | ... | ... | ... | ... | 1 | 3 | 2 | ... | ... |
| Rock-pools | ... | ... | ... | ... | ... | 3 | ... | 2 | 3 | 10 | 2 | 1 |
| Rock-pools in stream-beds | ... | ... | ... | ... | ... | ... | ... | ... | ... | 8 | ... | 4 |

Aedes (Aedimorphus) occidentalis n.sp.

Aedes apicoannulatus Edwards, *Trans. Roy. Soc. Trop. Med. and Hyg.*, Vol. XVI, p. 500.

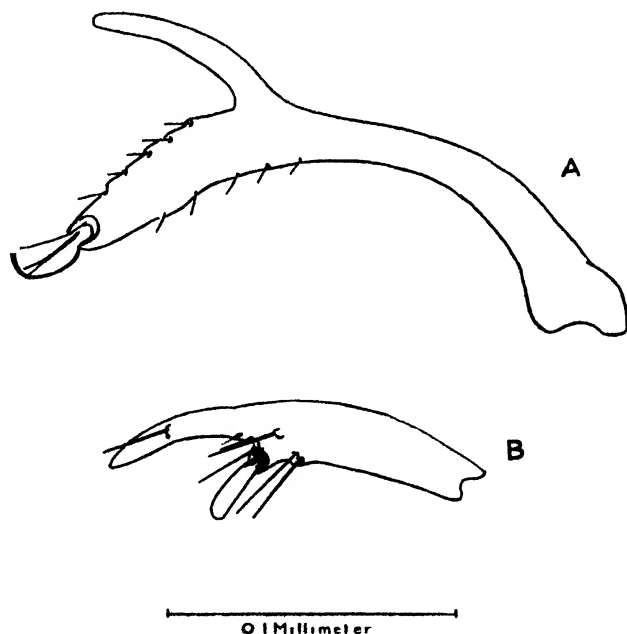
Ochlerotatus apicoannulatus Ingram and Macfie, *Bull. Ent. Res.*, Vol. VIII, p. 144.

It was found that amongst the large numbers of *Aedimorphus* with apically banded tarsi there were two distinct species in addition to *A. simulans*, N. and C., which has characteristic mesonotal spots of narrow, silvery scales. One of these species was entirely without white scales on the thorax; it was present in numbers enormously greater than the other species, which possessed antero-lateral stripes of flat white scales on the dorsal surface of the thorax. The side-pieces of the male hypopygium were quite distinct in the two species and the larvae exhibited striking differences. The less common species with the thoracic stripes has hitherto usually been identified with *apicoannulata* Edw., but Mr. Edwards, who kindly compared examples of both species with Theobald's type of *Aedimorphus alboannulatus* (Edwards bestowed the new name "*apicoannulata*" in 1912, *alboannulatus* being preoccupied), informed me that the commoner mosquito with unornamented thorax agreed with the type. The rarer species must, therefore, be regarded as new and the name *occidentalis* is proposed, as this species is the western representative of two closely-allied African species (Edwards, 1923, p. 500). As the characteristics of this mosquito have been referred to by Edwards (1923, 1925), a full description does not seem necessary. The new species differs from *A. apicoannulatus* Theo. chiefly as follows:—The proboscis is entirely dark scaled; there are large paired patches of broad silvery scales immediately behind the eyes; in *A. apicoannulatus* the pale scales in this position are very narrow and yellowish; the mesonotum is adorned with paired stripes of silvery scales on the anterior margins, extending backwards to the scutal angle, the stripes consisting in front of two layers of outwardly directed flat scales and, behind, of irregularly arranged flat scales directed obliquely backwards. Dark scales of mesonotum unmixed with paler ones; in *apicoannulata* there is an admixture of yellowish-brassy scales among the dark ones of the mesonotum, but no definite pattern formed by pale scales. The male hypopygium

shows striking differences from that of *apicoannulata*; the claspers have been figured by Edwards (1923), but they are illustrated here (fig. 1, B) for comparison with those of that species. The larva has been fully described by Ingram and Macfie (1917); the chief differences between it and the larva of *apicoannulata* are tabulated on p. 102. Type ♀ and two cotype ♂♂ reared from larvae found in tree-holes, Freetown, 6.VIII.25, by officials of the Sanitary Dept., Sierra Leone.

A. (Aedimorphus) apicoannulata Edw.

This species appears to be by far the commonest tree-hole breeding mosquito in Freetown, at any rate during the wet season. The male hypopygium differs from that of *A. occidentalis* n.sp., in the form of the claspers (fig. 1, A and B), and in the possession of rudimentary claspettes, partially fused with the internal surface of the side-pieces,



A.M.E.

FIG. 1. Clasper of male hypopygium. A—*A. apicoannulata*;
B—*A. occidentalis*.

and bearing two strong spines at their extremities. The clasper in *A. simulans*, N. and C., is not distinguishable from that of *apicoannulata*, but the claspettes of the former species bear hairs instead of spines.

Larva. Fourth stage.

Head. Antenna curved, with a sub-median tuft of five hairs, and shaft sparsely spinose. Mid frontal hairs plumose. Mental plate (fig. 2, D) with a median tooth and fourteen teeth on each side, of which the inner seven or eight are very small and close together. First and second segments of abdomen with very stout, plumose, multiple hairs laterally, that on the first with four or five, that on the second with three or four branches. Lateral combs (fig. 2, A, C) consisting of sub-triangular patches of very numerous

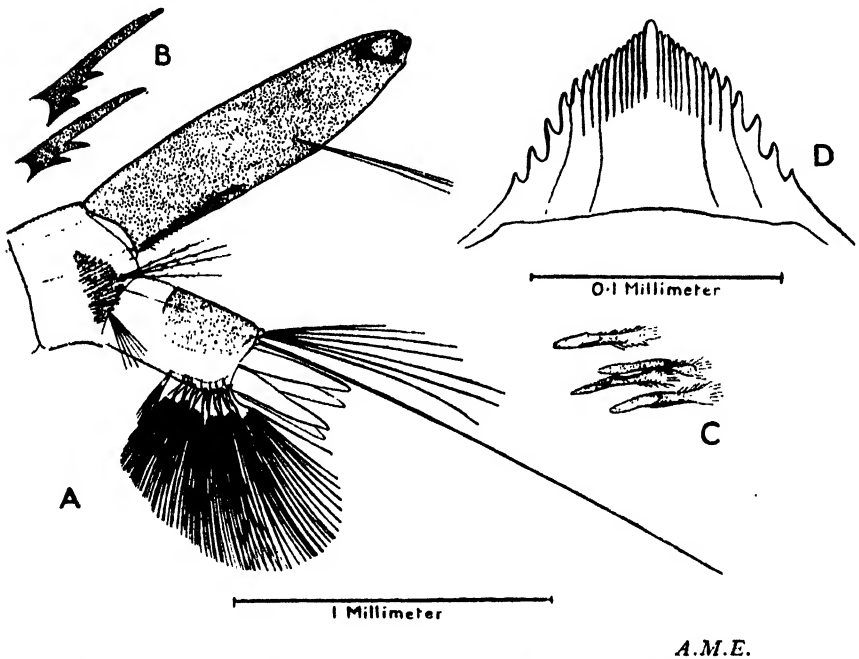


FIG. 2. *A. apicoannulata*, larva. A—eighth and ninth segments; B—pecten spines; C—comb spines; D—mental plate; B and C to the same scale as D.

(about eighty) small, elongate fringed spines. Siphon tube with the length four times the greatest width; pecten consisting of nineteen barbed teeth; tuft of two or three long hairs, reaching to or slightly beyond the apex of the siphon. Dorsal hairs of anal segment consisting of a tuft of seven hairs, and a hair arising from a separate base exceeding twice the length of the longest hairs of the tuft.

Table showing chief differences between the fourth stage larva of *A. apicoannulata* and *A. occidentalis*.

| | <i>A. apicoannulata</i> Edw. | <i>A. occidentalis</i> n.sp. |
|----------------------------------|----------------------------------|---|
| Mental plate | With 19 teeth | With 29 teeth |
| Lateral combs | 11-12 spines in an irregular row | About 80 spines in a sub-triangular patch |
| Tuft of siphon tube | 6 hairs | 2-3 hairs |
| Dorsal hairs of anal segment ... | 3 and 1 | 7 and 1 |

Megarhinus (Toxorhynchites) aeneus n.sp.

FEMALE. *Head*. Occiput with brilliant peacock-blue scales in front, and narrow, pale greenish scales with golden reflections behind and at the sides ; a border round the eyes narrow and purplish above, broader and white below. Proboscis and palpi with deep violet scales, those of palpi with bright blue reflections at the apices of the segments. *Thorax*. Prothoracic lobes with golden bristles and metallic blue scales with violet reflections above, and darker bristles and ochraceous golden scales below. Mesonotum with bright yellow setae in front, scales dull green appearing bronzy in certain aspects, brighter and more bluish-green scales on and near the scutellum and a small patch of bright blue scales over the wing root. Setae of scutellum and above wing root deep golden ; pleurae golden yellow with flat, white scales forming a broad median longitudinal band ; scales above and below this band yellow with pale green iridescence. *Abdomen*. Dorsum with first segment clothed with metallic green scales with yellow reflections, rest of segments with metallic violet-pink scales with coppery reflections, lateral edges of tergites with small basal areas of paler metallic scales ; sixth and seventh segments with small apical, lateral tufts of flame-coloured setae. Venter brilliant golden with pink and green reflections, seventh segment with broad median distal area of dark purplish-brown scales. *Legs*. All femora golden internally almost to the apex, and on the basal third or half externally, a line of golden scales extending on to the dark area for most of its length. Front tibia with dark metallic scales, middle tibia pale scaled on basal three-

fourths behind, hind tibia with a proximal line of pale greenish scales and a conspicuous patch of creamy-white scales at the outer third beneath. Front tarsi with lines of whitish scales beneath the first two segments, most conspicuous on the second; mid tarsi with white basal bands on the first two segments, that of the second being about two-thirds of its length; hind tarsi with sub-basal white band on the first segment, second segment entirely dark and third segment with a broad basal creamy-white band. *Wings*. Scales with metallic greenish reflections, posterior border with emargination well marked, length of wing about 5 mm.

Pupa. Respiratory trumpets with the opening deeper than in *M. brevipalpis* Theo. (Macfie and Ingram, 1923), the ratio of the length of the closed portion to that of the whole about 1 : 1.9. Paddles closely resembling those of *M. brevipalpis*. Macrochaetae of abdomen differing considerably from those of that species, lateral only present on fifth segment, projecting at right-angles; second segment with sub-lateral macrochaeta about equal in length to the segment behind; sub-median slightly shorter, a large trifid seta just internal to sub-median, its branches about equal in length to this seta. Third, fourth and fifth segments with sub-median about equal in length to the segments, sub-lateral considerably longer, that of the fifth extending beyond the distal border of the seventh segment; sixth segment with only sub-lateral represented, seventh and eighth segments without macrochaetae.

Type: one female bred from a larva taken from a tree-hole at Hill Station, Freetown, 29.vii.1925, by an officer of the Sanitary Department, Sierra Leone.

A second specimen bred from a larva taken from a hole in a Mango Tree at Hill Station, 11.vi.1924, differs in the amount of white on the legs. The front and mid tibiae are entirely dark-scaled and the hind tibiae have only a trace of the distal white patch. The front tarsi are without any pale scales and the middle tarsi have the white band on the second segment narrower than in the type. The pupal pelt shows considerable differences in the abdominal chaetotaxy, although it differs much more from *M. brevipalpis* in this respect; in the absence of a large series of specimens for comparison, it is impossible to say whether this represents a distinct species.

Anopheles smithii Theo. (1905) (Pl. VII).*A. (Feltinella) pallidopalpi* Theo. (1907).

The above synonymy which was put forward by Christophers (1924) in his 'Provisional List and Reference Catalogue of the Anophelini' has been confirmed by the examination of a large series of adults of both sexes reared from larvae collected at Mount Aureole, Freetown, by Professor Blacklock and the writer. The larvae, which are described by Blacklock and Evans in a paper published concurrently with this, are very characteristic, and show little variation. The adults, however, exhibit a striking dimorphism in the wing markings of the two sexes, the females having the pale spots so much reduced that the wings appear quite dark to the naked eye, while the wings of male specimens have well-developed *Myzomyia* spotting. The confusion to which this peculiarity has given rise is increased by the fact that in each sex the wing markings exhibit a striking degree of individual variation. A short account of the species with special reference to the wing markings is as follows:—

FEMALE. General colouration black; palpi with three narrow, rather obscure, pale bands; mesonotum clothed chiefly with hairs, scales being present only on the anterior promontory (Christophers, 1924) legs entirely black-scaled. *Wings*. The most extensively pale scaled specimens (Pl. VII, A) show small spots at the following points:—costa: just beyond the end of the sub-costa, at the apex and between these points; sub-costa: about mid-way between base and apex; first vein: near the base, opposite the sub-costal spot, opposite the sub-apical and (sometimes) apical costal spots; wing field: at the base of the fork cells; on the second, third, fourth and upper branch of fifth vein in the region of the cross-veins. This condition occurred in about one-third of the specimens; in others one or more of the pale spots were absent, but the suppression of some of the spots and retention of others was entirely promiscuous, and almost every possible variation of pattern was observed. In two examples pale scales were entirely absent and in several specimens only two or three obscurely pale scales were present. Spots may be reduced almost to extinction either by reduction of the number of pale scales involved or by being rather dusky so that the contrast

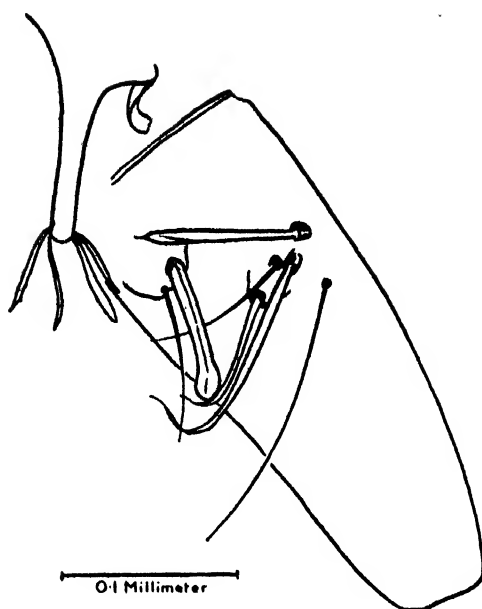
with the dark scales is not well-marked. Usually the two wings of the same specimen were of similar pattern.

Wing length : 2.8 to 3.5 mm.

MALE. The male is considerably lighter in appearance than the female owing to the extensive white scaling of the wings. The characters of the head, palpi, mesonotum, abdomen and legs did not show any marked variation and agreed with those of *A. pallidopalpi* as described by Theobald (1907). *Wings.* Theobald's description of the wing markings would apply to some of the specimens met with ; the majority of our specimens, however, had the basal border spot involving the costa as well as the first vein, a condition which Theobald noted in one of his specimens. Typical examples with well-developed spotting (Plate VII, B) showed, in addition to the three large white spots involving the costa and first vein, a small apical spot. There were also well-marked spots in the following situations :—first vein : towards the base and just beyond the first border spot (' accessory sector spot,' Christophers) ; at the bases of the fork cells ; at the cross-veins ; on the basal half of the third vein ; two extensive areas on the fifth vein and two smaller ones on its upper branch ; one on the sixth.

In two exceptional cases a large white area also occurred distally on vein three. Other specimens showed reduction of some of the pale areas, the reduction, as in the female, not following any definite plan but involving sometimes one, sometimes another spot or combination of spots. The paleness of the scales also varied greatly in intensity.

HYPOPYGIUM (fig. 3). Christophers (1925) refers to the *Myzomyia*-like character of the hypopygium in imperfectly displayed specimens in the British Museum, labelled *smithii* and *pallidopalpi*. Our material shows that three of the five parabasal spines are very broad, the outermost being almost blade-like.



A.M.E.

FIG. 3. *A. smitibii*. Side-piece and phallosome of male hypopygium.

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PLATE VII

EXPLANATION OF PLATE VII

Anopheles smithii Theo.

- A. Wing of female.
- B. Wing of male.



THE MOUTH PARTS, ALIMENTARY TRACT, AND SALIVARY APPARATUS OF THE FEMALE IN *PHLEBOTOMUS PAPATASII*

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PLATES VIII-XIV

The external morphology of the mouth parts of *Phlebotomus papatasii* has been described by Grassi (1907) and by Newstead (1911), but so far as we are aware, a detailed study of the biting, pumping and salivary apparatus has not been made for any member of the genus *Phlebotomus*.

The following study was made on *Phlebotomus papatasii*, the commonest sandfly of Palestine. The method of study adopted was observation of the mouth parts *in vivo*, dissection and serial sections, transverse, sagittal and coronal of the head.

Observation *in vivo* is the most satisfactory method of studying the action of the mouth parts. A sandfly is lightly anaesthetised with ether so that it remains motionless for about ten minutes, during which time the mouth parts are usually working actively, so that their movements can be observed under the microscope, and the contractions of the muscles of the buccal cavity can also be seen and counted. If the insects are stunned by shaking them vigorously in a test-tube, and their wings and legs removed, the movements of the external mouth parts and the contraction of the muscles of the buccal cavity will often continue up to four hours, during which period they can be conveniently studied.

Dissection of the mouth parts can be made in freshly-killed insects, but for permanent mounted preparations it is advisable to leave the insects overnight in a 5 per cent. solution of potash before dissecting.

Serial sections were made after fixation in Henning's solution or Dobell's fluid, as recommended by Hoare (1921).

There are several formulae for Henning's solution differing in the amount of nitric acid they contain; the following formula, taken from Bolles Lee (1921), was found useful for sandflies and mosquitos.

| | | | | | | |
|---|-----|-----|-----|-----|-----|----------|
| Nitric acid | ... | ... | ... | ... | ... | 16 parts |
| Chromic acid, 0.5 per cent. | ... | ... | ... | ... | ... | 16 parts |
| Saturated solution of mercuric chloride in 60 per cent. alcohol | ... | ... | ... | ... | ... | 24 parts |
| Saturated solution of picric acid in water | ... | ... | ... | ... | ... | 12 parts |
| Absolute alcohol | ... | ... | ... | ... | ... | 42 parts |

After fixation in Henning's solution for one day, the insects were placed for two hours in a mixture of equal parts Lugol's solution and 70 per cent. alcohol; they were then passed through changes of 70 per cent. alcohol till no trace of iodine remained. Some specimens were passed through alcohol and paraffin in the usual way and others were stained in bulk, either in a saturated solution of eosin in 70 per cent. alcohol for two days, or in haematoxylin for three days, and then treated in the usual manner. After fixation in Henning's solution for twenty-four hours, sections cannot be cut less than 20μ thick. For a study of the musculature of the mouth parts and head, sections 50μ thick are the most convenient. Staining in bulk for three days in Ehrlich's haematoxylin gives the best results for histological purposes after fixation in Henning's solution.

Dobell's process is very satisfactory for histological purposes, for after fixation in Dobell's fluid, sections 7μ thick can be obtained, but the process is a lengthy one and is not necessary for the study of the muscular system.

MOUTH PARTS

The proboscis consists of the labrum-epipharynx, hypopharynx, two mandibles, two maxillae and a labium. The length of the proboscis from the mouth to the tip of the epipharynx is 400μ . The armed parts when at rest lie unsheathed in the labium in the following manner: the labrum-epipharynx is superior and the two mandibles lie one above the other immediately between the epipharynx and hypopharynx; the maxillae lie beneath and lateral

to the hypopharynx and for the greater part of their course appear moulded to the inferior lateral aspect of the latter. It will be seen from Pl. IX, figs. 7 and 8, Pl. XIII, figs. 14 and 15, that when the parts are at rest the epipharynx and one of the mandibles form a canal, the roof of which is a groove on the inferior surface of the epipharynx, and the floor the upper surface of one of the mandibles. This canal is directly continuous with the buccal cavity and we have found it to contain *Herpetomonas* five days after feeding on an oriental sore.

The distal ends of the epipharynx, hypopharynx and mandibles lie at the extremity of the labium between the labella, and the distal ends of the maxillae lie a varying distance behind them. Owing to the disposition of the mandibles the teeth of the epipharynx and hypopharynx are never in contact when the insect is at rest.

The labrum-epipharynx is 400μ long. The labrum is a thin chitinous band which rises from the anterior superior end of the clypeus and is attached to the epipharynx first by a narrow strip of muscle and then by loose membranous tissue; it becomes fused to the epipharynx a little behind the distal end of the latter.

The epipharynx is 400μ long and 40μ at its broadest part; the middle of its lower surface is grooved by a channel which, in transverse section, is triangular, the apex of the triangle being rounded (Pl. IX, figs. 7 and 8, Pl. XIII, figs. 13-15); this channel forms the roof of the food canal during the act of feeding. The distal end of the epipharynx is pointed and toothed, each tooth being curved and pointing forwards, and its toothed margin is concave.

The hypopharynx is 400μ long and 40μ at its broadest part. It is pierced through the whole of its length by the salivary canal; its distal end is toothed, the teeth being smaller than those of the epipharynx, and the toothed margin is convex. The upper surface of the hypopharynx forms the floor of the food canal during the act of feeding.

The mandibles are 420μ long and 30μ in their broadest part, and rise from the clypeus above, behind, and lateral to the mouth. The base of the mandible is divided into two cornua, one external and one internal. Rising from the infero-lateral aspect of the clypeus is a sclerite (Pl. XI, fig. 2) which curves upwards and inwards and terminates in a free point at the side of the clypeus; the free end of this sclerite lies between the two cornua of the mandible (Pl. XI,

fig. 2) and plays an important part in regulating the movements of the latter. The cornua of the mandible are darkly pigmented and form a well-marked external feature of the cranium of *Phlebotomus papatasi*.

The mandible passes downwards and inwards for a short distance and the inner side of this portion shows a strongly-marked ridge into which the adductor muscle of the mandible is inserted. Beyond the adductor ridge the mandible turns inwards and passes straight downwards between the epipharynx and hypopharynx. The distal end of the mandible is sharply pointed and its inner margin is serrated for a distance of 60μ (Pl. XI, fig. 1). The tip of each mandible is seen at the opposite side of the proboscis distally (Pl. XIV, fig. 2).

The maxillae are composed of two parts, a blade which is extra-cranial and a long process which is intra-cranial. The blade is 330μ long and 30μ in its broadest part; at its distal end, externally, there are five or six tooth-like processes pointing backwards and gradually diminishing in size from before backwards; a part of the internal margin, 120μ long and commencing 35μ behind the distal end, is also armed with tooth-like processes which point forwards. The long process of the maxillae (Pl. XI, fig. 3) is a rod of chitin 325μ long which runs backwards along the floor of the cranium; in a cleared preparation of the head it appears lateral and inferior to the whole of the buccal cavity and a part of the pharynx; it is attached by a broad chitinous band to the floor of the cranium and the points of attachment on the two sides are connected by a thin strip of membrane (Pl. XI, fig. 3).

The labium, when at rest, is 420μ long and 110μ at its broadest part, i.e., at the level of the labella, and is, with the exception of several structures to be described later, a soft organ with membranous walls. The ventral and lateral surfaces form the mentum and the concave dorsal surface forms the labial gutter (Pl. IX, figs. 7 and 8), in which the armed parts are ensheathed when the insect is at rest.

The mentum rises from a horse-shoe shaped piece of chitin (Pl. XIV, figs. 3 and 4) which forms the anterior end of the gular region and serves as the origin of the intrinsic labial muscles. The labium itself contains, in addition to the muscles, a large amount of fat, two main tracheae and their branches and the two labial nerves which rise from the middle of the lower border of the inferior ganglion

of the brain. The cavity of the labium is connected by a wide opening with the body cavity. The two labella are articulated by a chitinous disc to the labium. A median lobe or glossa, according to Grassi's terminology, lies between the two labella. The structure of the labella and glossa can only be suitably studied in fresh preparations when the whole labium is contracting and the labella become separated and the glossa distended.

When the labium is at rest the median ends of the chitinous discs which form the base of the labella are almost in contact (Pl. XIV, fig. 3), the glossa is almost completely hidden from view and its details have therefore been overlooked in previous descriptions.

On the ventral side the median end of the base of the labellum is articulated through a small rod of chitin (Pl. XIV, fig. 1) to an elongated chitinous rod which passes inwards and backwards to unite with its fellow from the other side (Pl. XIV, figs. 1, 3 and 4), and form a support for the ventral side of the labium.

On the median half of the dorsal surface of the labella are a number of fine, closely-set lines or grooves running almost horizontally (Pl. XIV, fig. 1) which produce the appearance of a pseudo-tracheal membrane. Near the distal ends of each labellum, dorsally, are four pre-stomal teeth, three median, which are close together, and one individual tooth external and more distal than the others (Pl. XIV, fig. 1). A minute muscle is inserted into the base of each tooth.

Inside each labellum near the distal end there is a group of nerve cells.

Ventrally each labellum is divided into a proximal and a distal half by a fine chitinous line (Pl. XIV, fig. 1) which, after pursuing a horizontal course till it almost reaches the internal border of the labellum, passes backwards to join the median end of the base of the latter. On the distal half of the labellum, ventrally, are a number of long and rather stout hairs.

The glossa (Pl. XIV, figs. 1, 2 and 4) is a transparent structure divided into two by a median longitudinal line; running through the greater part of each half is a thick chitinous line which is attached proximally to the median end of the base of the labella (Pl. XIV, fig. 1); from this chitinous line others radiate and the glossa folds up along these chitinous lines when the labium returns to the resting position. Distally the dorsal surface of the glossa is finely corrugated.

The palps have been amply described by Newstead, who noted that the fourth and fifth segments are bent downwards and backwards in such a way that the palps protect the proboscis. The third segment extends slightly in front of the proboscis. In Palestine *P. papatasi* shows an interesting local variation from the same species as described by Newstead, from Malta. In Malta, Newstead found that the fourth and fifth segments are distinctly annulated; in Palestine the second and third segments are also distinctly annulated.

THE MUSCULATURE OF THE MOUTH PARTS AND THE METHOD OF BITING

In anaesthetised specimens of freshly-killed sandflies it is seen that the greater part of the gular region moves backwards and forwards in one plane. The part of the gular region that is involved in this movement is separated by a fold of membrane from a narrow area which extends in front of the occipital foramen and is bounded latero-posteriorly by the narrow inferior openings of the two intra-cranial tunnels.

During retraction the fold between the motile part of the gular region and the narrow area in front of the occipital foramen deepens and the membranous floor of the motile portion bulges downwards.

The intra-cranial tunnels are two hollow chitinous rods (Pl. XI, fig. 4), one on each side, which are connected with the exterior, superiorly and anteriorly, by a wide funnel-shaped opening in front of the base of the first antennal segment, and inferiorly and posteriorly by a narrow opening at the level of the posterior margin of the eye. Anteriorly they are connected by transverse chitinous tubes which unite in the middle line and are produced backwards as a chitinous bar which, after a short distance, branches into two chitinous bands which fuse with the roof of the cranium (Pl. XI, fig. 4). The superior ends of the intra-cranial tunnels serve as an origin for the intra-cranial muscles to the first antennal segment.

The muscles responsible for this movement are: (1) a pair of powerful protractor muscles (Pl. XII, fig. 1) which rise, one on each side, from the roof of the clypeus and passing downwards and backwards are inserted into the posterior part of the movable

portion of the gular region ; (2) a pair of retractor muscles, one on each side, which rise partly from the floor of the cranium immediately behind the inferior opening of the intra-cranial tunnel and partly from the lower part of the intra-cranial tunnel, and are inserted into the extreme anterior end of the gular region (Pl. XII, fig. 1).

The labium, maxillae and palps are attached to the gular region and they are, therefore, carried backwards and forwards during the similar movements of the latter.

The maxillae and palps have, in addition, muscles peculiar to themselves. The maxilla is supplied by a muscle which rises from the roof of the clypeus behind the protractor of the gular region and passes downwards and forwards to be inserted into the long process of the maxilla a little behind the junction of the latter with the blade (Pl. XII, fig. 2) ; thus although receiving directly only the insertion of one muscle, the maxilla is acted upon by three muscles, owing to its attachment to the floor of the cranium, and of all the armed mouth parts the maxilla has directly and indirectly the largest and most powerful supply of muscles.

From the posterior part of the floor of the movable portion of the gular region a muscle arises which passes forwards and slightly upwards to be inserted into the first segment of the palps and acts as an elevator and abductor of the palps. During the act of feeding the palps are elevated and abducted.

The mandibles are supplied by two relatively powerful muscles (Pl. XII, fig. 2) which rise from the posterior part of the cranium laterally and inferiorly and pass forwards and slightly upwards. The internal and narrower of these two muscles (Pl. XII, fig. 2, mm₁) is inserted into the adductor ridge of the mandible, and the external and broader muscle (Pl. XII, fig. 2) is inserted into the tip of the external cornu. The external muscle, shortly before its insertion into the external cornu, turns round an invagination into the side of the clypeus and passes outwards for a short distance ; this muscle abducts and rotates the mandible externally. The internal muscle pursues a straight course forwards and medianwards towards insertion and, on contraction, causes adduction and internal rotation of the mandible. When the mouth parts are in action the mandibles are rapidly abducted and adducted through a narrow angle and are at

the same time rotated, movements which can be readily followed *in vivo* under the microscope. Abduction and adduction are both limited by the wedge of chitin which lies between the two cornua, for when the mandible is abducted through a narrow angle the external cornu is pressed against the wedge of chitin and further abduction is impossible; when the mandible is adducted through a narrow angle the internal cornu is pressed against the wedge of chitin and further adduction is impossible. When the mandibles are in action it is seen that the cornua move in an arc round and in front of the wedge of chitin lying between them; the mandibles, as judged by the movements of the cornua, also undergo a limited amount of backward and forward movements owing to the elasticity of the sclerite from which they rise. During the act of biting the inner dorsal margin of the expanded labella also imposes a limit to the abduction of the two mandibles.

The epipharynx and labrum are supplied by laterally symmetrical muscles, two of which function as regulators of the diameter of the food canal during the act of feeding (Pl. XII, fig. 1).

These muscles are: (1) a muscle which arises from the commencement of the labrum and, passing downwards and slightly forwards, is inserted into the epipharynx; (2) a muscle which arises from the roof of the clypeus posteriorly and, passing obliquely downwards and forwards, is inserted into the junction of the epipharynx and roof of the buccal cavity.

A third muscle arises from the anterior part of the roof of the clypeus and, passing through the origin of the labrum, is inserted into the under surface of the latter.

We are now in a position to understand the rôles played by the various armed mouth parts in biting. The maxillae, mandibles, epipharynx and hypopharynx all act as piercing stylets, but the most important rôle is played by the maxillae, for they have the widest range of movement backwards and forwards, and directly and indirectly possess the most powerful muscle supply of all the biting parts. The mandibles, through their rapid movements of adduction, abduction and rotation, combined with the small backward and forward movements described above, penetrate and enlarge the wound in all directions.

Patton and Cragg (1913) are of the opinion that the epipharynx

and hypopharynx do not play the part of active piercing stylets in the mosquito during the act of biting, but it is doubtful whether this view holds in the case of *Phlebotomus papatasi*. We have observed two males of *P. papatasi* containing blood, one of them gorged with fresh blood actually leaving a human being; in both cases the mouth parts were characteristic of the normal male, i.e., the mandibles were absent and the maxillae unarmed. It is interesting to note that in both cases dissection showed the genitalia, external and internal, to be of the normal male type and no trace of hermaphroditism was found.

During the act of feeding the mandibles no longer interpose between the epipharynx and hypopharynx and the two latter coming into opposition form the food canal; their teeth interlock and probably act as a strainer, preventing particles of too large a size from entering the food canal.

The labium undergoes interesting changes during the act of biting. There are two sets of intrinsic longitudinal muscles in the labium, all arising from the chitinous base of the mentum. An external longitudinal muscle is formed by two bellies which arise from the outer part of the base and unite into one tendon which is inserted into the lateral margin of the union of the body of the labium and the base of the labellum (Pl. XIV, fig. 4). Since the labium is a lax structure composed mainly of soft tissues, the external longitudinal muscle causes a decrease of length and an increase of breadth of the whole labium. The internal longitudinal muscle is composed of four bellies which rise from the median part of the base of the labium and pass downwards; at the level of the base of the labella they unite to form a single tendon which passes outwards through the labella and is inserted into the distal end of the latter (Pl. XIV, fig. 4). Contraction of this muscle causes abduction and expansion of the labella. Abduction of the labella is also secured indirectly by the action of the external longitudinal muscle of the labium, for the latter, by increasing the girth of the labium, causes distention and abduction of the labella. As the labella are abducted the median lobe or glossa comes into view; the glossa expands and opens out along the chitinous rays described above in the manner of a fan opening out. When the labium returns to the normal resting position the glossa again folds up. The labium when contracted by the action

of its intrinsic muscles is up to 120 μ shorter than the resting labium and it is still further shortened by pressure against the skin ; the biting parts projecting beyond the labella have thus ample room for piercing the skin and reaching the blood capillaries.

When the armed mouth parts are in action they lie on the expanded glossa and the median dorsal margin of the labella forms the walls of a groove, guides the mouth parts and restricts the range of their action laterally.

The above description of the movements of the mouth parts of *Phlebotomus papatasi* applies in general principles to the movements of the mouth parts of *P. minutus* and *P. perniciosus* and they may, therefore, be considered as characteristic of the genus *Phlebotomus*.

Phlebotomus papatasi usually bites during the night and early morning but occasionally, under natural conditions, also bites during the day ; and in the laboratory specimens which have been starved several days frequently bite and feed readily by day. In our experience about 60 per cent. of specimens under laboratory conditions refuse to feed under any circumstances and die of starvation. Specimens in which the eggs are ripe or nearly ripe usually refuse to feed. *P. papatasi* often bites several times before feeding and we have observed one specimen bite seven times on an area of skin half-an-inch in diameter before feeding. There is an interval of fifteen to thirty seconds between the commencement of the act of biting and the entrance of blood into the buccal cavity ; aetiologically this interval is important for it gives an opportunity for parasites in the proboscis to enter the wound. When blood is already flowing into the buccal cavity the negative pressure caused by the muscles of the latter would tend to prevent parasites from the food canal entering the wound.

The buccal cavity is formed by the union of the continuation backwards into the cranium of the epipharynx and hypopharynx ; it is composed of three chitinous plates, an inferior one which forms the floor of the cavity and is strongly chitinised, and two lateral plates which meet in the mid-line and are feebly chitinised except at their lateral margins. The continuation of the hypopharynx inside the cranium splits into two laminae, a superior one which is strongly chitinised and convex ventrally, and which forms the floor of the buccal cavity, and an inferior one which is continuous posteriorly

with the inferior part of the common salivary duct (Pl. X, fig. 2). The salivary pump lies between these two laminae.

It is difficult to give an exact verbal description of the shape in transverse section of the buccal cavity, for this differs at various points and can be best appreciated from the figures of transverse sections of the clypeus at various levels (Pl. IX, fig. 6, Pl. XIII, figs. 8-12).

It will be seen that the lumen of the buccal cavity when at rest is very narrow and of a peculiar shape, roughly triangular with the base of the triangle thick and slightly convex downwards, and the sides of the triangle extremely concave inwards.

The lateral part of the backward continuation of the epipharynx is, in marked contrast to the median portion, strongly chitinated, and forms a strong bar of chitin which is fused to the lateral margin of the floor of the buccal cavity. This bar passes upwards and backwards (the general direction of the buccal cavity) and splits into two bars, one inferior and one superior. The inferior bar (Pl. X, figs. 2 and 3) proceeds backwards and then turns downwards and passes below the floor of the buccal cavity, and meeting its fellow from the opposite side forms an arch convex backwards. This arch forms a support for the buccal cavity and during the whole of its course (Pl. XII, fig. 3) serves as an origin for a relatively large and powerful salivary muscle which, converging from all points of the arch, passes downwards and forwards to be inserted into the salivary pump (Pl. VIII, fig. 1, Pl. XII, fig. 3). The upper bar passes upwards and backwards and terminates in a cornu on each side, the two cornua being united by two cross-pieces, one anterior and the other posterior (Pl. X, fig. 3). The connecting tube between the buccal cavity and the pharynx lies below the two cross-pieces.

The buccal cavity is supplied by a large group of muscles which rise from the roof of the clypeus near the middle line and are inserted into the two chitinous plates which form the roof of the buccal cavity. The general direction of these muscles is downwards and forwards, but a few longer than the others pass downwards and backwards from the roof of the clypeus and are inserted into the most posterior part of the buccal cavity (Pl. XII, fig. 4).

The action of the muscles of the buccal cavity can be studied *in vivo*, when they are seen to contract up to one hundred and twenty times a minute; they act as pumping organs, for by creating a

negative pressure they pump blood into the buccal cavity through the food canal. When the muscles contract the two superior chitinous plates are pulled upwards and outwards and the buccal cavity is thus dilated (Pl. XIII, fig. 10).

Immediately behind the buccal cavity and lying below the two cross-pieces is a small chitinous tube 30μ long which joins the buccal cavity to the pharynx. This tube is surrounded by a sphincter muscle which regulates the flow of blood from the buccal cavity into the pharynx (Pl. VIII, fig. 1, Pl. XI, fig. 4),

The pharynx is 210μ long and 63μ in its broadest part; it is broad posteriorly and narrow anteriorly. It is composed of three chitinous plates, one superior and horizontal and two lateral. The transverse section of the lumen of the pharynx varies at different levels but it is roughly triangular in shape, the base of the triangle being superior and all three sides concave internally. Posteriorly ridges are seen on the wall of the pharynx; these are the optical expression of internal teeth which extend for a distance of 80μ along the lateral walls and for a slightly shorter distance along the superior wall. The foremost teeth are small and point backwards and the remainder are vertical (Pl. XII, fig. 4).

The pharynx, except for a small portion posteriorly and another anteriorly, lies inside the brain.

The pharynx is supplied by the following bilaterally symmetrical muscles:

(1) A dorsal anterior group of muscles which rise from the roof of the cranium immediately behind the clypeus and pass downwards to be inserted into the superior plate of the pharynx. The anterior fibres pass vertically downwards and the posterior ones obliquely downwards and backwards between the superior ganglion of the brain and the pharynx (Pl. VIII, fig. 1, Pl. XII, fig. 4).

(2) A dorsal posterior group of muscles which rise from the roof of the cranium above and in front of the occipital foramen and pass downwards to be inserted into the superior plates of the pharynx. The anterior fibres of this group pass obliquely forwards and downwards between the superior ganglion of the brain and the pharynx (Pl. VIII, figs. 1 and 2, Pl. XII, fig. 4).

(3) A powerful group of muscles which rise from the infero-lateral aspect of the cranium posteriorly and pass upwards and forwards to

be inserted into the inferior plates of the pharynx. The anterior fibres of this group pass obliquely upwards and forwards between the pharynx and the inferior ganglion.

Although the greater part of the pharynx lies inside the brain, yet owing to the peculiar direction of the muscles almost the whole surface of the pharynx serves as an insertion for dilator muscles.

The function of the above-described muscles is to pump blood from the buccal cavity into the pharynx, the short tube lying between the pharynx and buccal cavity acting as a regulator of the flow of blood.

The oesophagus is a short tube 80 μ long, as measured from the posterior opening of the pharynx to the commencement of the midgut. It is attached for a considerable distance to the sides of the pharynx and thus a pouch is formed between the external wall of the pharynx and the oesophagus (Text-fig. 1). This pouch has been found to contain *Herpetomonas*. The wall of the oesophagus is lined by a single layer of epithelium which lies on a basal membrane; the interior surface of the epithelium is covered with a very fine layer of chitin.

The oesophageal diverticulum lies ventral to the midgut; it opens into the oesophagus at a varying distance from the posterior end of the latter; exceptionally it opens into the pharynx together with the oesophagus. The diverticulum is composed of two very fine layers of muscle fibres, one longitudinal and internal and the other circular and external; internally it is lined with flat epithelium covered by a thin layer of chitin. There is a very narrow sphincter at the junction of the oesophagus and diverticulum. In freshly dissected insects the diverticulum is usually seen to be undergoing peristaltic movements towards the oesophagus. Unlike the oesophageal diverticulum of mosquitos the diverticulum of *P. papatasi* seldom contains air bubbles. Out of four thousand sandflies examined only one instance was observed of an air bubble in the diverticulum and this in spite of the fact that the midgut often contains air bubbles.

Waterston (1922) states that blood can be seen in the oesophageal diverticulum for about forty-eight hours after a meal, while Patton and Cragg (1913) state that the oesophageal diverticulum is filled with blood immediately after a feed but is empty several hours later. In our experience, based on a dissection of four thousand sandflies, it is unusual to find blood or bloodstained fluid in the

oesophageal diverticulum at any time, and in the few cases where blood is found it is present only in negligible quantities as compared with the amount found in the midgut. Even when the insect is fully gorged and the stomach distended to its fullest capacity, the

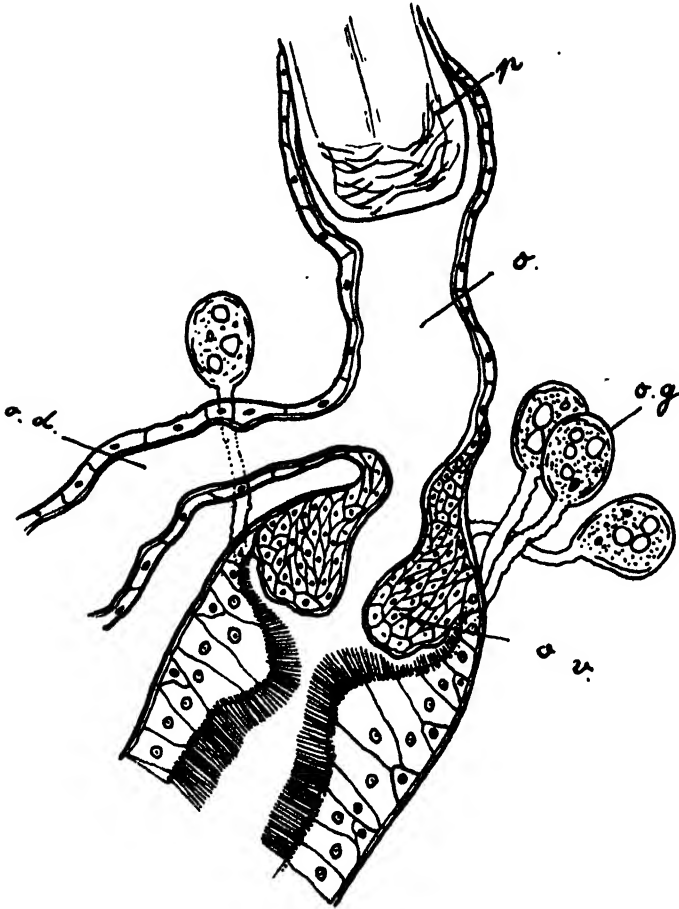


FIG. 1 (diagrammatic). *p.*—pharynx; *o.*—oesophagus; *o.d.*—oesophageal diverticulum; *o.g.*—oesophageal glands; *o.v.*—oesophageal valve.

Note rods projecting from the epithelial cells of the cardia.

Note pouch between the sides of the cardia and the valve.

oesophageal diverticulum contains very few red cells or none at all. Out of twenty-five sandflies killed immediately after a feed four contained red cells in the oesophageal diverticulum, but only in small quantities. In insects killed a few days after a feed

the oesophageal diverticulum is usually found distended with perfectly clear fluid. This fluid is later either absorbed through the thin walls of the diverticulum or is passed into the midgut. In specimens kept after feeding until the midgut is empty, the oesophageal diverticulum is usually also empty. It appears that the oesophageal diverticulum may function as a reservoir, but only for fluids and not for red cells.

There are four small elliptical oesophageal glands 23μ long and 18μ broad, which contain large yellow granules; they lie round the commencement of the midgut and each gland opens by a minute duct into the pouch between the oesophageal valve and the midgut (Text-fig. 1).

The midgut consists of two parts which differ histologically. The upper part or cardia is tubular and lies in the thorax; it is lined by a single layer of columnar epithelium of peculiar structure; near the internal surface of each cell there are a number of fine granules and from each granule a thin transparent rod projects into the lumen of the gut (Text-fig. 1). The rods somewhat resemble cilia but they are not motile or contractile; they are closely packed and in fresh undamaged preparations they are not very evident, but if the wall of the gut is broken by pressure and individual cells set free, their true nature can be readily determined. Each rod is 7.5μ to 10.5μ long. Lying between the epithelial cells near their base there are a number of small interstitial cells.

The oesophageal valve lies at the superior end of the cardia and consists of a downward projection of the oesophagus into the cardia; between this projection and the wall of the midgut there is a small pouch (Text-fig. 1 and Pl. VIII, fig. 2). The posterior surface of the pouch is lined with epithelium characteristic of the cardia.

The structure of the cardia bears an interesting relation to the development of *Herpetomonas* in sandflies. Christophers, Shortt and Barraud (1925) found large numbers of *Herpetomonas* attached to the epithelium of the upper part of the midgut in a number of specimens of *Phlebotomus argentipes* fed on a case of Kala-azar, and the authors have recorded a natural infection of *Phlebotomus papatasi* with *Herpetomonas* in which the parasites were attached to the posterior surface of the oesophageal valve. The authors, in a series of experiments in which one hundred and fifty-five specimens

of *P. papatasi* were fed on oriental sores, found that in nine out of sixteen positive cases flagellates attached themselves to the wall of the cardia, in some cases as early as the third day. The parasites can be seen boring into the epithelium with their flagella, and when they are completely attached their flagella lie entangled among the rods projecting from the epithelial cells and even reach into the cytoplasm. Division of the flagellates takes place mainly in the cardia, particularly in the anterior end which is found in artificial and in some cases in natural infections, to be completely choked up by a mass of flagellates.

The cardia is not distensible, in marked contrast to the second part of the midgut, i.e., the stomach.

The stomach lies in the abdomen and its condition depends on the amount of blood contained and the time from the last feed; it is lined by a single layer of epithelium which does not contain the rods characteristic of the cardia.

The stomach is very distensible and accommodates itself to the relatively enormous feeds of *P. papatasi*. An average female of *P. papatasi* weighs 0.3 milligrams and, after a full feed, weighs 0.4 milligrams. (It is interesting to note that *P. papatasi* never passes blood per rectum during the act of feeding.)

Digestion is a relatively slow process. It is not uncommon to find erythrocytes in a good state of preservation four days after a feed, and in one instance we observed unimpaired erythrocytes eight days after a feed. Haemolysis does not take place as a rule till the third or fourth day after a feed.

A day or two after feeding the distention of the stomach is diminished and the oesophageal diverticulum is filled with a colourless fluid, and during this period no blood is passed in the faeces nor are erythrocytes destroyed in the stomach. Unaltered haemoglobin is never found in the epithelial cells of the stomach but it is passed in the faeces. The above observations tend to show that the essential food element in the blood is the plasma and not the red cells and this is further supported by the fact that sandflies will feed when the red cells from the previous feed are still present in the stomach.

The epithelium of the midgut contains a powerful anticoagulin. In five experiments two small scratches were made close together on

human skin ; the empty midgut of *P. papatasii* was dissected out and rubbed into one scratch and the other was left untreated. Blood oozed from the scratch into which the midgut was rubbed for a considerably longer time, in one instance three-quarters-of-an-hour, than from the other.

The red cells do not come in direct contact with the epithelium of the stomach, for in *P. papatasii* (also in *P. minutus* and *P. perniciosus*) a very definite peritrophic membrane is produced. The peritrophic membrane is readily seen in specimens killed a day or two after a feed ; it is a thin white amorphous structure which contains the mass of red cells. Anteriorly it extends into the cardia and posteriorly into the hindgut. The peritrophic membrane may be likened to a sealed tube closed anteriorly and posteriorly.

The Malpighian tubes rise near the posterior end of the stomach by two common ducts, as described and figured by Newstead (1911). Near their origin the ducts divide into two tubes each about $1,300\mu$ long and 24μ thick ; these extend downwards almost to the end of the abdomen and then curve backwards, their distal extremities lying in the upper part of the abdomen near the thorax.

Immediately behind the origin of the Malpighian tubes there is a ring of muscles which marks off the midgut from the hindgut and serves as a point of origin for the peristaltic movements of the latter. The wall of the hindgut consists of two layers of muscles, one longitudinal and external and the other oblique and internal. The lumen is lined by a single layer of cubical epithelium, the inner aspect of which is covered with an exceedingly thin glistening chitinous layer. In the posterior part of the hindgut are two large rectal papillae 30μ long and 20μ in their widest part ; these are composed of large polygonal cells with a small round nucleus. The rectal papillae are richly supplied with tracheae.

THE SALIVARY APPARATUS

The salivary apparatus consists of the salivary glands, salivary ducts, salivary pump and the salivary channel through the hypopharynx.

The salivary glands lie one on each side in the uppermost ventral part of the thorax. They are hollow, almost spherical organs lined

with a single layer of columnar epithelium which rests on a basal membrane. A fully-distended salivary gland may reach the size of 180μ long by 140μ wide. Immediately after a feed the salivary glands are small and the epithelium thin. If a series of sandflies is dissected at various times after a feed it is seen that the epithelial cells become progressively larger and filled with granules. After a time cells are found free in the lumen of the gland, which contains, in addition, fine granules secreted by the epithelium. The free cells degenerate and break up into refractile granules much larger than those secreted by the epithelium. Three or four days after a feed the gland is distended with secretory granules and the products of degeneration of liberated cells and the epithelium lining the gland is thinned by pressure (Text-fig. 2, *a-e*). It will be seen from the above description that the saliva is composed of the products of liberated cells which degenerate in the lumen and of the secretion of the cells lining the lumen of the gland. Generally there is a parallelism between the condition of the salivary glands and the condition of the stomach.

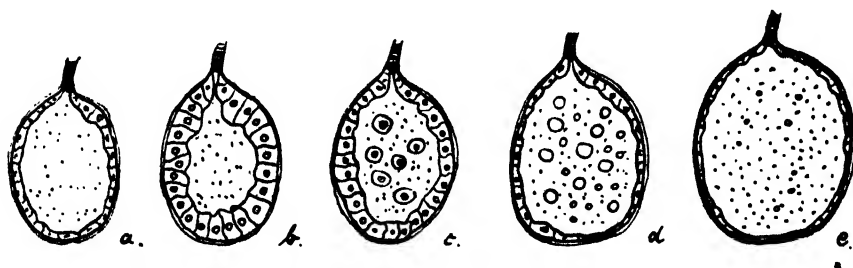


FIG. 2 (diagrammatic). Salivary glands in various stages. *a*.—immediately after a feed; *b-e*.—subsequent stages.

Note in *c*. free cells in the lumen.

Sandflies which had been kept in the laboratory for four days without food, so that digestion was well advanced and the salivary glands large, were dissected. Twenty salivary glands were placed in 20 cmm. neutral distilled water, the glands were broken up with fine needles and the resulting emulsion transferred to a capillary tube of narrow bore into which a minute amount of phenol red was drawn. The emulsion showed a faint alkaline reaction.

A series of glands were emulsified in 20 cmm. saline and various

amounts of blood were added. The mixtures were drawn into fine capillary tubes and the coagulation time compared with controls containing the same amount of saline and blood. It was found that an emulsion of eight distended salivary glands in 20 cmm. saline delayed coagulation of 2 cmm. human blood for fifteen minutes, and an emulsion of twelve salivary glands delayed the coagulation of 2 cmm. human blood for thirty minutes. (Controls showed complete coagulation in nine minutes.) Since, in nature, the contents of two salivary glands are used for not more than 0.1 cmm. blood, as compared with 0.5 cmm. and 0.3 cmm. in the above experiment, it follows that the saliva functions as an anticoagulant during the act of biting.

Emulsions of ten and twenty salivary glands in 20 cmm. of a 1 per cent. solution of sodium citrate in saline were mixed with 5 cmm. blood and the mixture drawn up in fine capillary tubes and observed under the microscope; no haemolysis and no agglutination took place during an observation period of six hours.

The salivary ducts are annulated tubes with thin chitinous walls 140μ long and a lumen 7.5μ wide (strikingly wider than the lumen of the salivary ducts of mosquitos which are 2.5μ wide). They pass into the head and converge to the middle line where they unite to form the common salivary duct. The common salivary duct is 190μ long and 11μ wide and has the same structure as the salivary ducts; it passes in the middle of the head underneath the inferior ganglion of the brain and opens into the salivary pump. The inferior wall of the common salivary duct shortly before its entrance into the salivary pump joins the inferior lamina of the hypopharynx.

The salivary pump is elliptical, 100μ long by 45μ wide (Pl. X, fig. 2, and Text-fig. 3). Its walls are formed of thick chitin and are traversed interiorly by strongly-marked circular ridges. A little in front of the entrance of the common salivary duct the floor of the salivary pump contains a small yellow elevation from which a number of minute teeth project into the lumen of the pump. Anteriorly the lumen of the salivary pump is continuous with the salivary canal which pierces the hypopharynx.

We have to thank Mr. M. Ber, of Jerusalem, for collecting a large number of sandflies and for being the subject of numerous experiments.

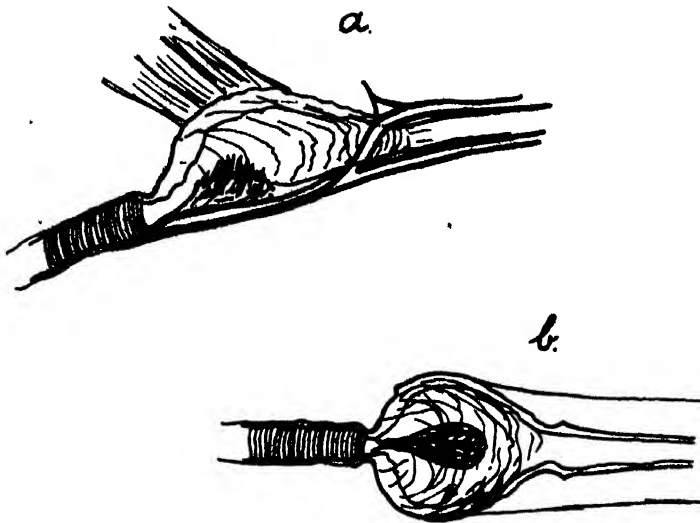


FIG. 3 (diagrammatic). Salivary pump. *a.*—side view; *b.*—dorsal view.

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ADDENDUM

There are usually five prestomal teeth on each labellum, three set close together as described and figured above and two more distal than the others.

Since the above paper was written we have observed one instance of *P. papatasii* passing a minute amount of fluid per anum during the act of feeding but this is very exceptional and therefore not of aetiological importance.

(According to Parrot (1922) *P. minutus* var. *africanus* passes fluid per anum during feeding.)

Parrot (1922) also observed an interval between biting and the entrance of blood into the insect (temps préparatoire) ; the interval noted was three to six minutes in *P. minutus* var. *africanus* and a half to one minute in *P. papatasii*.

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15 Jan., 1926

EXPLANATION OF PLATE VIII

Fig. 1. Sagittal section through the middle of the head of
P. papatasi. . $\times 100$.

b.m.—Muscles of the buccal cavity.

s.g.—Superior ganglion of the brain.

i.g.—Inferior ganglion of the brain.

c.s.d.—Common salivary duct.

s.m.—Salivary muscle inserted into salivary pump.

h.—Hypopharynx.

l.e.—Labrum-epipharynx.

Fig. 2. Sagittal section through pharynx and oesophageal valve.
 $\times 130$.

o.—Oesophagus.

o.d.—Oesophageal diverticulum.

o.v.—Oesophageal valve.

p.t.—Pharyngeal teeth.

Fig. 3. Connecting tube between pharynx and buccal cavity.
 $\times 150$.

c.t.—Connecting tube.

s.—Sphincter muscle.



FIG. 1



FIG. 2



FIG. 3

EXPLANATION OF PLATE IX

Transverse sections through the head and the proboscis.
(All figures $\times 300$.)

Fig. 1. Pharynx at its commencement.

Fig. 2. Pharynx showing teeth.

Fig. 3. Pharynx : the two lateral plates only are toothed.

Fig. 4. Pharynx immediately before entering the brain.

Fig. 5. Pharynx in the middle of the brain.

Fig. 6. Section through the clypeus.

s.p.—Salivary pump.

b.c.—Buccal cavity. Floor and sides of the buccal cavity are thickly chitinised ; the roof is thinly chitinised.

Figs. 7 and 8. Sections through the proboscis.

The sections are arranged progressively from behind forwards.



FIG. 1



FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6

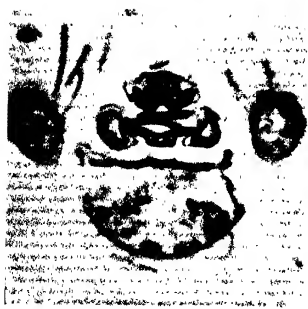


FIG. 7



FIG. 8

EXPLANATION. OF PLATE X

Fig. 1. Salivary glands.

Fig. 2. Lateral view of buccal cavity.

c.s.d.—Common salivary duct.

s.p.—Salivary pump.

el.—Elevation on the floor of the salivary pump from which minute teeth project.

Fig. 3. Dorsal view of the pharynx and the buccal cavity.

(All figures $\times 225$.)

b.c.—Buccal cavity.

p.—Pharynx.

i.b.—Chitinous arch beneath the buccal cavity from which the salivary muscle rises.



FIG. 1



FIG. 2



FIG. 3

EXPLANATION OF PLATE XI

Fig. 1. Mandible.

e.c.—External cornu.*ab* .—Abductor tendon of the mandible.*adc.*—Adductor tendon of the mandible.*i.c.*—Internal cornu.*r.*—Ridge into which the adductor tendon is inserted.

Fig. 2. Origin of the mandible.

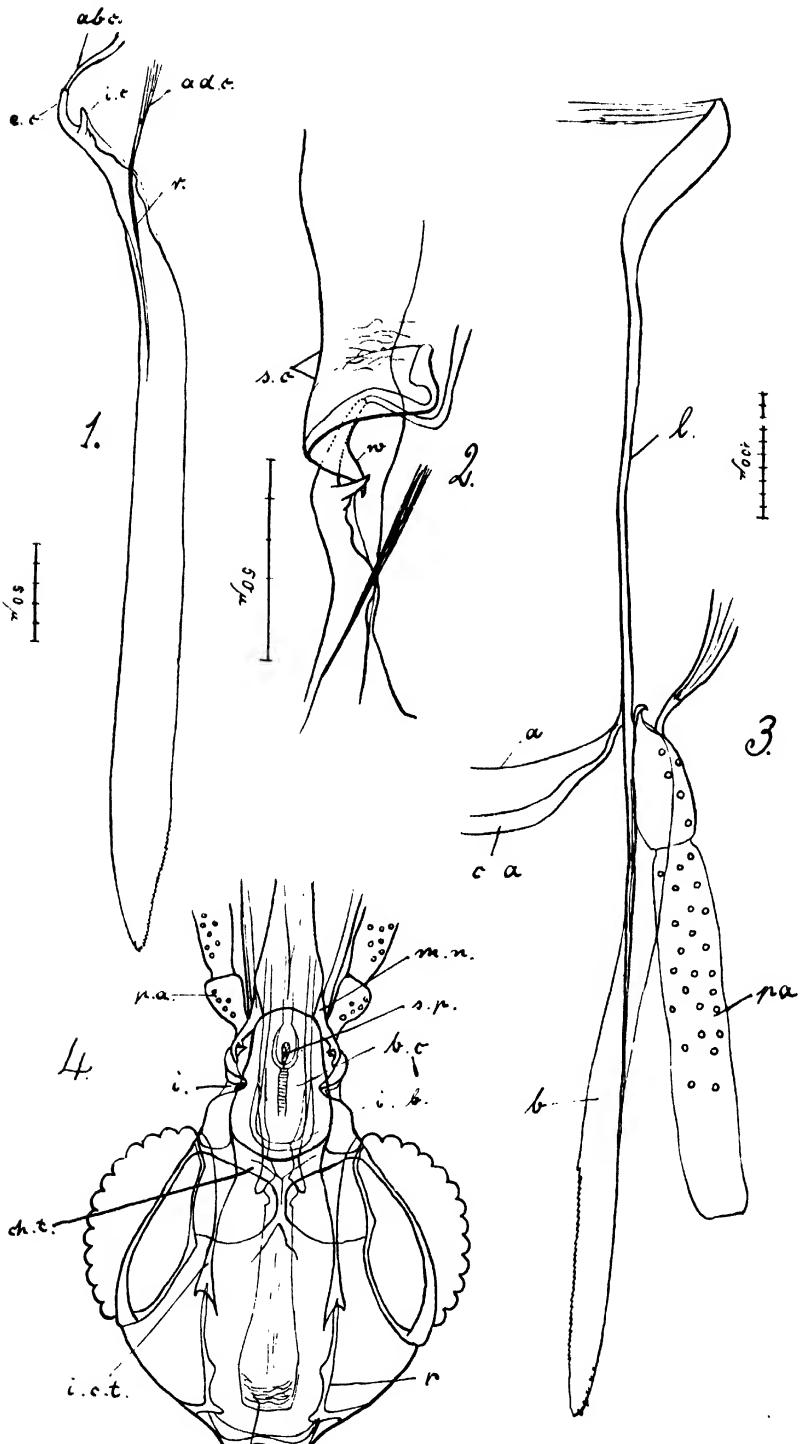
sc.—Sclerite from which the mandible arises.*w.*—Wedge of chitin between the two cornua.

Fig. 3. Maxilla.

l.—Long process (intracranial).*b.*—Blade.*a.*—Anterior margin of the clypeus.*c.a.*—Chitinous arch at anterior part of the gular region from which the mentum arises.*pa.*—Palp.

Fig. 4. The head as seen in a cleared preparation from above.

i.c.t.—Intracranial tunnel.*ch.t.*—Chitinous tubes uniting the intracranial tunnels anteriorly.*p.*—Pharynx.*b.c.*—Buccal cavity.*i.b.*—Chitinous arch beneath the buccal cavity.*s.p.*—Salivary pump.*r.*—Ridge in front of the occipital foramen.*i.*—Invagination into the sides of the clypeus round which the abductor tendon of the mandible turns.*mn.*—Mandibles.*pa.*—Palp.

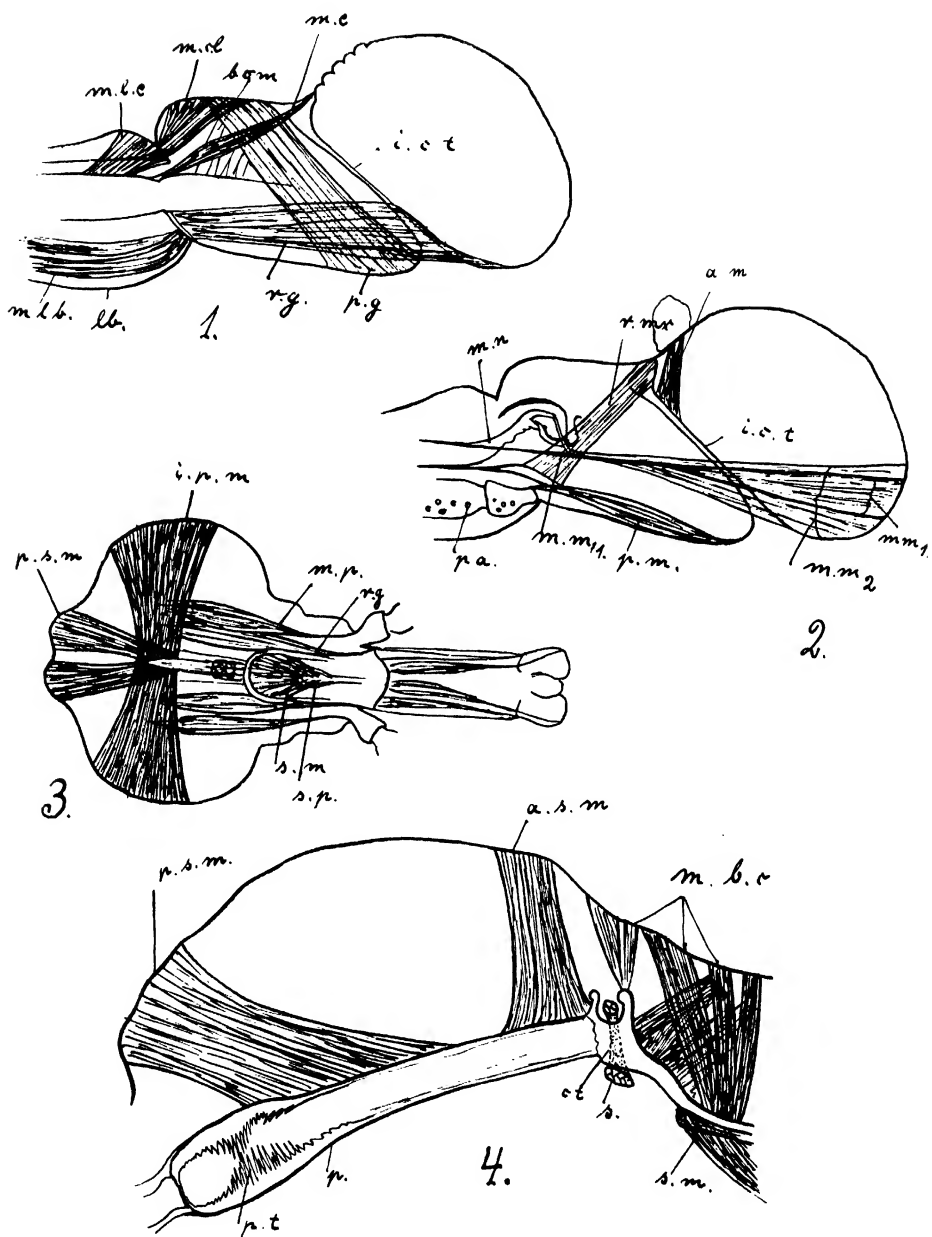


EXPLANATION OF PLATE XII

The muscles of the head (semi-diagrammatic).

- Fig. 1. *p.g.*—Protractor muscle of the movable part of the gular region.
r.g.—Retractor muscle of the movable part of the gular region.
b.c.m.—Muscles to the buccal cavity.
m.l.e.—Muscles from the labrum to the epipharynx.
m.c.l.—Muscles from the clypeus to the labrum inserted into the under surface of the labrum.
m.e.—Muscle from the posterior part of the roof of the clypeus inserted into the epipharynx at the junction of the latter with the roof of the buccal cavity.
i.c.t.—Intracranial tunnels.
l.b.—Labium.
m.lb.—Intrinsic muscles of the labium.
- Fig. 2. *r.mx.*—Retractor muscle of the maxilla.
m.m.₁—Adductor muscle of the mandible.
m.m.₂—Abductor muscle of the mandible.
p.m.—Palpal muscle.
mn.—Mandible.
pa.—Palp.
a.m.—Antennal muscles.
- Fig. 3. *p.s.m.*—Posterior superior pharyngeal muscles.
i.p.m.—Inferior pharyngeal muscles.
m.p.—Palpal muscle.
r.g.—Retractor muscle of the gular region.
s.m.—Salivary muscle.
s.p.—Salivary pump.
- Fig. 4. *p.*—Pharynx.
p.t.—Pharyngeal teeth.
a.s.m.—Anterior superior pharyngeal muscles.
p.s.m.—Posterior superior pharyngeal muscles.
c.t.—Connecting tube between the buccal cavity and the pharynx.
s.—Sphincter muscle round the connecting tube.
m.b.c.—Muscles of the buccal cavity.
s.m.—Salivary muscle.

In figs. 1 and 2 the inferior opening of the intra-cranial tunnels marks the posterior limit of the movable part of the gular region.

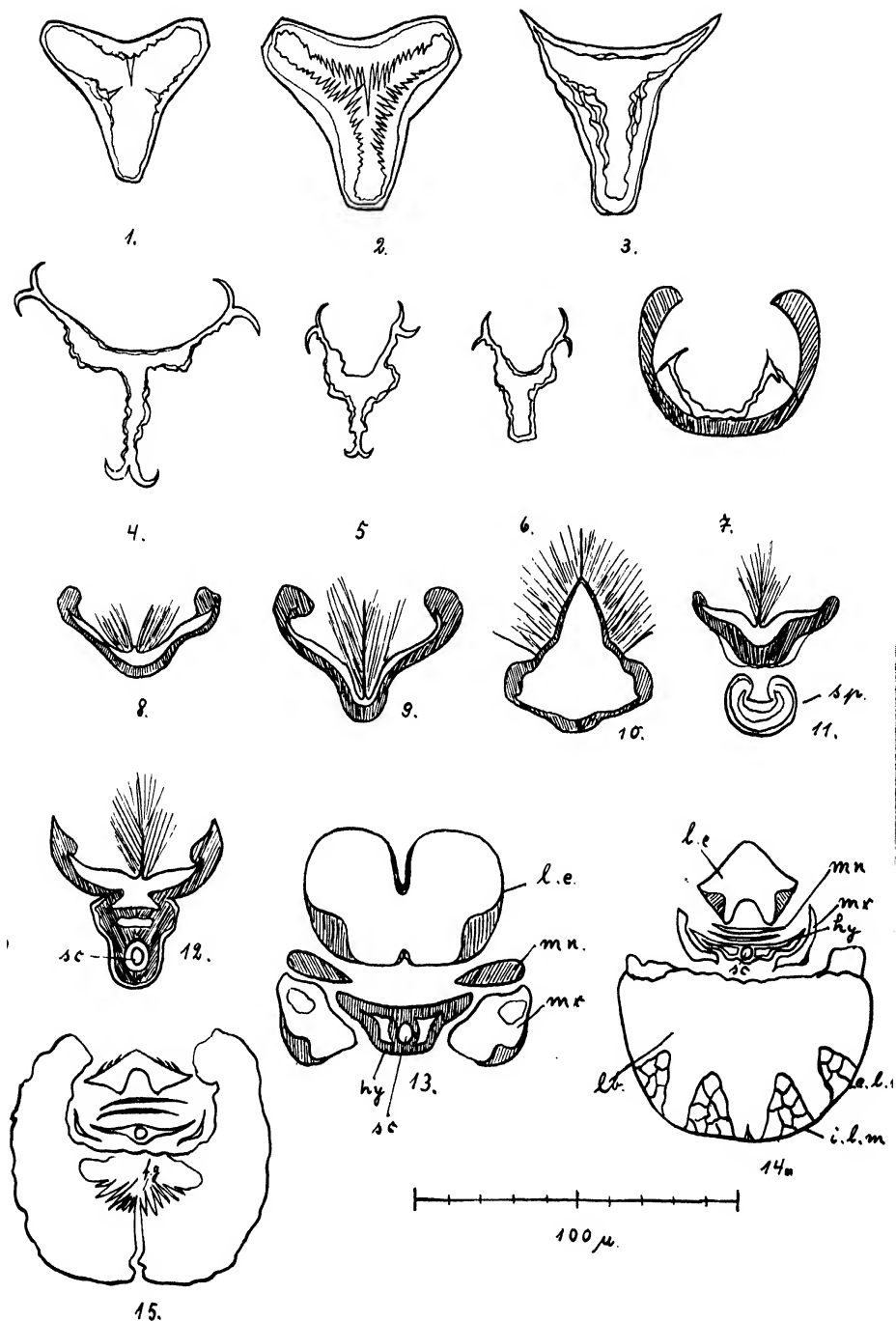


EXPLANATION OF PLATE XIII

Transverse sections through pharynx, buccal cavity and proboscis at various levels from behind forwards.

(Camera-lucida drawings.)

- Figs. 1 to 4. Pharynx before it enters the brain.
- Fig. 5. Pharynx in middle of brain.
- Fig. 6. Anterior end of pharynx.
- Fig. 7. Connecting tube between pharynx and buccal cavity.
- Figs. 8 to 9. Buccal cavity.
- Fig. 10. Buccal cavity dilated.
- Fig. 11. Buccal cavity and salivary pump (*s.p.*).
- Fig. 12. Buccal cavity in front of salivary pump.
s.c.—Salivary canal.
- Fig. 13. Commencement of proboscis.
l.e.—Labrum-epipharynx.
mn.—Mandible.
mx.—Maxilla.
hy.—Hypopharynx.
s.c.—Salivary canal.
- The mandibles do not yet interpose completely between the epipharynx and hypopharynx.
- Fig. 14. Section through the proboscis.
lb.—Labium.
e.l.m.—External longitudinal muscle of the labium.
i.l.m.—Internal longitudinal muscle of the labium.
- Fig. 15. Section through the proboscis near the tip.
f.g.—Folds of glossa.



A CASE OF POROCEPHALOSIS

BY

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(Received for publication, 17 December, 1925)

PLATE XV

The following case is of interest because of the rarity of the condition ; the parasite has not been previously reported from the Anglo-Egyptian Sudan.

Patient was a man aged 36 years (Zehei Bakheit), and lived some thirty miles from Khartoum ; he was a Sudanese.

He died in the Khartoum Civil Hospital on the 11th October, 1925.

POST-MORTEM FINDINGS

At the post-mortem examination, a condition of advanced tuberculosis of the lungs with pleural effusion was found. The tubercular nature of the lesion was later confirmed by microscopical sections which showed a condition of typical broncho-pneumonic tubercle.

There were several encysted larvae of *Porocephalus armillatus* in the surface of the liver (Pl. XV, fig. 1) and also eight similar cysts in the submucosa of the duodenum.

No free nymphae were noticed in the peritoneal cavity, and no encysted larvae were found in the lungs.

PATHOLOGY

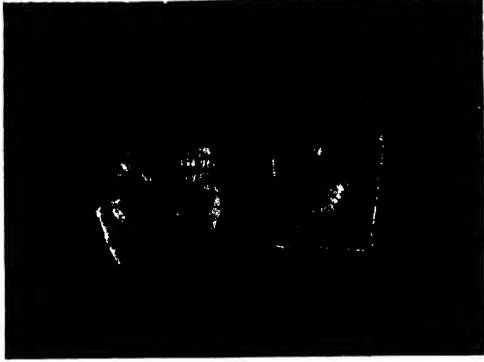
The parasite was certainly not the immediate cause of death ; the possibility that the destructive action of the parasite in the lungs predisposed to the development of the tubercular infection was considered, although no encysted larvae were found in these organs.

KHARTOUM,

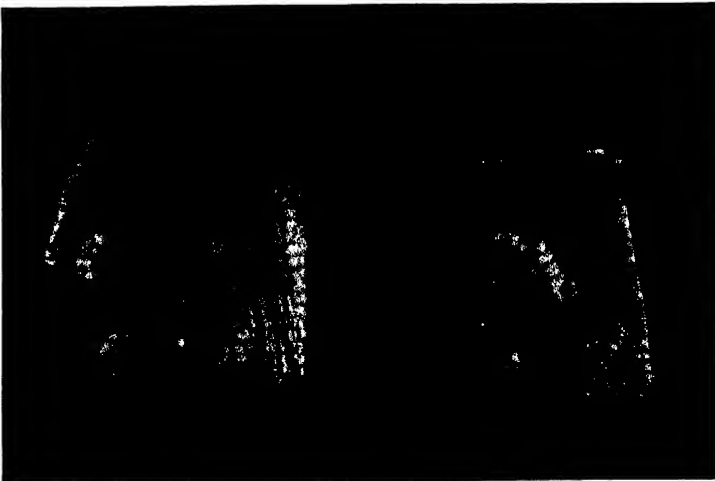
3rd December, 1925.

EXPLANATION OF PLATE XV

- Fig. 1. Photograph of larvae in surface of liver (natural size).
The larva consists of sixteen segments and is $1\frac{1}{2}$ cms.
long, by 3 mm. diameter (cross section).
- Fig. 2. (Enlargement). Shows larva curled up in its transparent
chitinous capsule, with its head towards the centre of
the circle.



1



2.

MISCELLANEA

PLASMODIUM REICHENOWI

BLACKLOCK AND ADLER, 1924

This name was given by Blacklock and Adler to a crescent-forming parasite of the chimpanzee found by them in Sierra Leone ; they concluded as the result of cross infection experiments that it was not identical with *P. falciparum*. From a personal communication of Dr. Swellengrebel to Dr. Bagshawe, Director of the Tropical Diseases Bureau, it appears that Swellengrebel and Ihle have previously applied the name *Laverania reichenowi* to this parasite in 1922, on page 12 of 'Sluiter, Swellengrebel and Ihle. De dierlijke parasieten van den mensch en van onze huis dieren, 3d. Ed. Amsterdam, 1922. Scheltema and Holkema.'

B. BLACKLOCK.

ON A COLLECTION OF ACANTHOCEPHALA IN THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

T. SOUTHWELL

AND

J. W. S. MACFIE

(Received for publication 11 March, 1925)

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The examination of this small collection of Acanthocephala has led us to attempt a tentative classification of the numerous genera hitherto described. The classification is based largely on the published descriptions of various authors which, unfortunately, are sometimes incomplete in details, a knowledge of which would

have been of great assistance, since the simplified morphology of the *Acanthocephala* offers at best but few characters on which to base a classification, and even these are liable to variation. Our work has therefore been one of great difficulty, and the result leaves much to be desired. To classify the group satisfactorily it will be necessary to obtain a much larger collection of species than we have had at our disposal, and a more extensive knowledge of the life history of the various worms.

Amongst the somewhat unsatisfactory characters upon which it has been necessary to base classification, mention should be made of the following :—

(1) *Lemnisci*. Even in mature worms, the length of the lemnisci appears (at least in certain genera) to vary within rather wide limits ; the length also varies, of course, with age ; and, moreover, the length relative to the total length of the body varies somewhat with the state of contraction or relaxation of the worm. From a systematic point of view, therefore, account must be taken of the age of the specimen and of the degree to which it is contracted.

(2) *Testes*. In young worms the shape, size, and relative position of the testes may be quite different from what they are in the adult. Reference has been made to this fact in the description of *M. moniliformis*. The degree of contraction of the body may also alter to some extent the position of the testes in the body and their relationship to each other, and this should be taken into account in those cases in which the position of the testes is of systematic importance.

(3) *Prostatic glands*. No reliance can be placed on the appearance of the prostatic glands of young worms. In mature worms it is frequently extremely difficult to determine the number of prostatic glands, but as some authors attach great importance to it, we have been unable to avoid employing it as a diagnostic character (see *ECHINORHYNCHIDAE*). Moreover, our experience has convinced us that the shape and arrangement of the prostatic glands are by no means constant, and as diagnostic characters must not be pressed too far, only differences of considerable degree being significant.

(4) *Eggs*. Eggs taken from the body cavity may or may not be fully developed and therefore it is clearly unwise to describe the eggs from specimens obtained in this manner. We have frequently

observed notable differences to exist between the more and the less mature eggs in a single worm. As the characters of the eggs are occasionally of importance, however, and as usually the only eggs available for examination are those taken from the body of the worm, it is important to select for description none excepting those which appear to be mature, namely, those in which the three concentric membranes are clearly defined, and the ring of hooks on the embryo developed. As an aid to the recognition of mature eggs we may say that, so far as our experience goes, the embryos in them are of a brownish colour.

With reference to the retractibility of the proboscis, a distinction must be drawn between a retraction of the entire proboscis or 'proboscis-like structure' within the anterior part of the body, and a retraction (invagination) of the proboscis within its sheath. In this paper a reference to the proboscis as being retractile means that it is capable of being invaginated into its sheath.

As we employ certain terms in a sense in which they are not used uniformly by other authors the following definitions must be given :—

(1) *Proboscis*. The proboscis, as usually understood, signifies the process at the anterior extremity of the body which is used as an organ of fixation, and which (excepting in *Apororhynchus hemignathi*) is armed with hooks. We consider that this structure is not always morphologically identical, and therefore we propose to limit the term 'proboscis' to that part of the process at the anterior extremity of the body which lies anterior to the insertion of the proboscis-sheath, and to use the term 'proboscis-like structure' when referring to the proboscis as understood colloquially. We cannot agree with Lühe and Van Cleave (1916) in considering this unreasonable because it involves the admission that in the genus *Gigantorhynchus* there is little or no true proboscis. On the contrary, we regard it as characteristic of the genus *Gigantorhynchus* that the proboscis is reduced, and maintain that the morphology of the 'proboscis-like structure' of *G. echinodiscus*, and the forms of the hooks with which that structure is armed, afford strong support to the view that in this species the true proboscis is represented by only the one or two circles of large hooks at the anterior extremity.

(2) *Body*. We define the anterior limit of the body as being situated at the level of the insertion of the lemnisci. This is, of course,

a purely arbitrary definition which, however, we consider necessary for systematic purposes.

(3) *Neck*. Considerable importance is attached to the presence or absence of a neck in the Acanthocephala, and to the presence or absence of hooks on this neck, but there does not appear to us to be any general agreement as to what constitutes a neck, some authors using the term to indicate a zone, often devoid of hooks, at the base of the 'proboscis-like structure,' and others using it in a more restricted sense. We therefore propose to define the neck as being that part of the worm which lies between the base of the proboscis and the anterior extremity of the body, that is, between the level of the insertion of the proboscis-sheath and the level of the insertion of the lemnisci. Thus in the genus *Echinorhynchus* the proboscis, using the term in its colloquial sense, is entirely or almost entirely the true proboscis, in the genus *Gigantorhynchus* it is largely neck, whilst in the genus *Centrorhynchus* it is approximately half true proboscis and half neck.

Classification.—Westrumb, in 1821, briefly reviewed the earliest observations made on the Acanthocephala. The order **Acanthocephala** was established by Rudolphi, in 1809, the following being the characteristics assigned to it by him in 1819:—'Corpus teretiusculum, utriculare, elasticum. Proboscis seriatum uncinata retractilis. Individua alia mascula, alia feminea.' Rudolphi recognised one genus only, namely, *Echinorhynchus*, with the characters of the order. Diesing, in 1851, accepted Rudolphi's classification, recognising only the single genus *Echinorhynchus*, but considered the order **Acanthocephala** to be a tribe which he included in the sub-order **Aprocta**.

Cobbold, in 1879, erected the family ECHINORHYNCHIDAE to accommodate the single genus *Echinorhynchus*, but did not define its characters; and Leuckart, in 1886, used the family name ACANTHOCEPHALIDAE without stating either the characters of the family or the genera he proposed should be included in it, but apparently for the reception of the single genus *Echinorhynchus*.

The first important attempt to split up the Acanthocephala was made by Hamann, who, in 1892 and 1895, divided them into three families as follows:—

(1) ECHINORHYNCHIDAE. Body elongated, smooth. Proboscis-

sheath with double walls ; the proboscis-sheath receives the proboscis. Nerve ganglion in the proboscis-sheath, generally in its depth, centrally placed. Hooks chitinised only at their tips, and with a root-like process below.

Genus *Echinorhynchus* ; with the characters of the family.

(2) GIGANTORHYNCHIDAE. Large species with a segmented, flat, taenia-like body when alive. Hooks like those of *Taenia*, being entirely covered with chitin, and with two root-like processes. Proboscis-sheath muscular, inserted into the proboscis, and into which the proboscis cannot be retracted. Nerve ganglion situated behind the middle of the proboscis-sheath, lying laterally and eccentrically. The body cavity lined by a structureless membrane and traversed by oblique membranes. Lemnisci long coiled tubes with a central canal.

Genus *Gigantorhynchus* ; with the characters of the family.

(3) NEORHYNCHIDAE. Species which become sexually mature in the larval state. Proboscis-sheath a tube with a simple wall. In the skin, and in the lemnisci, are a few giant nuclei. Circular muscles very simply developed ; and the longitudinal muscles only present here and there.

Genus *Neorhynchus* ; with the characters of the family.

Since the publication of Hamann's classical work numerous authors have contributed to our knowledge of this interesting group of parasitic worms, amongst whom especial mention should be made of Lühe, Porta, Van Cleave, and Travassos.

The tentative classification which we propose is as follows. The species which we have had at our disposal are indicated in the body of the paper.

| | |
|---------------|---|
| Phylum | NEMATHELMINTHES. |
| Order | ACANTHOCEPHALA. |
| Sub-order (1) | Neoechinorhynchiea , nom. nov. |
| Family (1) | NEOECHINORHYNCHIDAE Van Cleave, 1919. |
| Genera | <i>Neoechinorhynchus</i> Stiles and Hassall, 1905. <i>Tanaorhamphus</i> Ward, 1918. <i>Octospinifer</i> Van Cleave, 1919. <i>Gracilisentis</i> Van Cleave, 1919. <i>Pandosentis</i> Van Cleave, 1920. |

- Family (2) QUADRIGYRIDAE Van Cleave, 1920.
 Genus *Quadrigyrrus* Van Cleave, 1920.
- Family (3) APORORHYNCHIDAE Shipley, 1900.
 Genus *Apororhynchus* Shipley, 1900.
- Sub-order (2) **Gigantorhynchiea**, nom. nov.
- Family (1) GIGANTORHYNCHIDAE Hamann, 1892.
 Genus *Gigantorhynchus* Hamann, 1892.
- Family (2) OLIGACANTHORHYNCHIDAE, nom. nov.
 Genera *Macracanthorhynchus* Travassos, 1917.
Oligacanthorhynchus Travassos, 1915.
Prosthenorchis Travassos, 1915.
- Sub-order (3) **Echinorhynchiea**, nom. nov.
- Family (1) RHADINORHYNCHIDAE Travassos, 1923.
 Genera *Rhadinorhynchus* Lühe, 1911.
Leptorhynchoides Kostylev, 1924.
Arhythmorhynchus Lühe, 1911.
Serrasentis Van Cleave, 1923.
Telosentis Van Cleave, 1923.
- Family (2) CENTRORHYNCHIDAE Van Cleave, 1916.
 Genera *Centrorhynchus* Lühe, 1911.
Mediorhynchus Van Cleave, 1916.
Empodius Travassos, 1916.
- Family (3) CORYNOSOMIDAE, nom. nov.
 Genera *Corynosoma* Lühe, 1904.
Bolbosoma Porta, 1908.
Polymorphus Lühe, 1911.
Filicollis Lühe, 1911.
Tegorhynchus Van Cleave, 1920.
- Family (4) MONILIFORMIDAE Van Cleave, 1924.
 Genus *Moniliformis* Travassos, 1915.
- Family (5) ECHINORHYNCHIDAE Cobbold, 1879.
 Genera *Prosthorhynchus* Kostylev, 1916.
Oligoterorhynchus Monticelli, 1914.
Pomphorhynchus Monticelli, 1905.
Acanthocephalus Koelreuter, 1771.
Echinorhynchus Zoega, 1776.

PHYLUM NEMATHELMINTHES.

Order *ACANTHOCEPHALA*.

Nemathelminthes without a gut, and with a proboscis-like structure which is usually armed with hooks.

With three sub-orders.

KEY TO THE SUB-ORDERS OF THE ORDER ACANTHOCEPHALA.

1. Prostatic glands a single syncytial mass..... *Neoechinorhynchidea* (1)
 Prostatic glands not a single syncytial mass.....2
2. Proboscis reduced, not capable of being withdrawn
 into the proboscis-sheath..... *Gigantorhynchidea* (2)
 Proboscis well developed and capable of being with-
 drawn into the proboscis-sheath..... *Echinorhynchidea* (3)

Sub-order I. **NEOECHINORHYNCHIDEA**, nom. nov.

Proboscis usually short and sub-spherical. Proboscis-sheath (when present) a tube with a simple wall. Prostatic gland a single syncytial mass. Nuclei of sub-cuticle and lemnisci few and very large.

The order is divided into three families.

KEY TO THE FAMILIES OF THE ORDER NEOECHINORHYNCHIDEA.

1. With a proboscis armed with hooks.....2
 Without such a proboscis..... *Apororhynchidae* (3)
2. Body bearing spines on the anterior region..... *Quadrigyridae* (2)
 Body devoid of spines..... *Neoechinorhynchidae* (1)

Family (1) **NEOECHINORHYNCHIDAE** Van Cleave, 1919

Neoechinorhynchidea of small to medium size. Wall of proboscis-sheath a single layer of muscle. Central nervous system near base of proboscis-sheath. Body devoid of spines; spines or hooks on proboscis only. Nuclei of sub-cuticle and lemnisci extremely large, normally of fixed number and definite arrangement, the sub-cuticle with five in the mid-dorsal line of the body and one in the mid-ventral line near the anterior end, and the lemnisci with two in one lemniscus and a single one in the other. Testes elliptical, usually contiguous. Prostatic gland a single syncytial mass containing relatively few

giant nuclei. Eggs where known with three membranes, and without polar capsules. Parasitic* in fish and reptiles (turtles).

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY NEOECHINORHYNCHIDAE.

1. Proboscis armed with 3 circles of hooks.....2
 Proboscis armed with more than 3 circles of hooks.....3
 2. Proboscis armed with 3 circles of 6 hooks each..... *Neoechinorhynchus* (1)
 Proboscis armed with 3 circles of 8 hooks each..... *Octospinifer* (3)
 Proboscis armed with 3 circles of 12 hooks each..... *Gracilisentis* (4)
 3. Proboscis several times longer than wide, armed with
 about 16 to 20 longitudinal rows each composed of
 about 10 hooks..... *Tanaorhamphus* (2)
- Proboscis short, cylindrical, armed with about 22
 longitudinal rows each composed of about 4 hooks... *Pandosentis* (5)

With regard to the last two genera, Van Cleave (1923) states in his key to the genera of Acanthocephala that in the genus *Tanaorhamphus* the proboscis bears 'twenty or more circles of hooks,' and in *Pandosentis* 'eight circles of hooks.' We are unable to harmonise these statements with his earlier generic definitions which we give below.

Genus (1) *Neoechinorhynchus* Stiles and Hassall, 1905.

SYNONYMS :—*Echinorhynchus* Zoega, in Müller, 1776, in part.
Neorhynchus Hamann, 1892, preoccupied.
Eorhynchus Van Cleave, 1914.

Diagnosis.—*Neoechinorhynchidae* with short, globose proboscis armed with three circles of six hooks each. Terminal hooks conspicuously larger and heavier than those of remaining rows, and* the only ones which bear conspicuous reflexed root-like processes. Each root a broad, flattened disc pyriform in surface view, usually approximately parallel to surface of proboscis wall. The thorn or hook proper attached at the apical or anterior end of the root, and appreciably longer than the root. Parasitic in fish and turtles.

Type species : *N. rutili* (Müller, 1780).

A single species belonging to this genus was found in the collection. This appeared to be a new species and is briefly described below.

* Unless otherwise stated the hosts given in this paper are those in whose alimentary canal the adult worms are found.

Neoechinorhynchus magnus, sp.n.

One immature female specimen only ; host unknown. Townsville, Queensland, Northern Australia. (Dr. P. A. Maplestone).

The specimen measured 90 mm. in length, and the maximum breadth was about 1.5 mm. The body is flattened and tape-like, the anterior extremity being much narrower than the posterior extremity ; the skin is slightly corrugated.

Proboscis. The proboscis is small, sub-globular, and armed, as is usual in the genus, with eighteen hooks in three rows, the anterior six being larger than the rest. The hooks of the terminal circle measure in length from 60μ to 71μ , those of the middle circle 30μ to 37μ , and those of the basal circle about 18μ .

Proboscis-sheath. This measures 0.5 mm. in length and the greatest breadth is 0.2 mm.

Lemnisci. These are slightly unequal in length and measure from five to six times the length of the proboscis-sheath.

The species differs from all other species of the genus in being very much longer.

Genus (2) *Tanaorhamphus* Ward, 1918.

SYNONYM :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—*Neoechinorhynchidae* of small to medium size, with cylindrical proboscis several times longer than wide. Proboscis armed with about sixteen longitudinal rows of hooks. Rows frequently incomplete and imperfect. Prostatic gland of the type characteristic of the family. Parasitic in fish.

Type species : *T. longirostris* (Van Cleave, 1913).

Genus (3) *Octospinifer* Van Cleave, 1919.

Diagnosis.—Proboscis short, globose, usually slightly broader than long ; provided with three circles of eight hooks each. Hooks of terminal circle not much larger or stronger than hooks of middle circle and but little longer than the root-process. Testes elliptical, in contact with each other but not joined by a broad contact-surface. Prostatic gland not in direct contact with posterior testis. The two lemnisci dissimilar in nuclear content, one possessing two giant nuclei and the other a single one. Central nervous-system located at one side of the proboscis-sheath, near its base. Parasitic in fish.

Type species : *O. macilentus* Van Cleave, 1919.

Genus (4) *Gracilisentis* Van Cleave, 1919.

SYNONYM :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—*Neoechinorhynchidae* of small size. Body proper unarmed. Proboscis provided with three circles of twelve hooks each. Each hook ensheathed in a prominent cuticular collar which permits only a small portion of it to protrude from the surface of the proboscis. Each hook of the terminal circle provided with a conspicuous root-process several times longer than the exposed portion of the spine. Root composed of a broad flat basal area which, by gradual diminution in size anteriorly, makes an ill-defined transition from thorn to root. Basal region of terminal roots frequently slightly indented. Hooks of middle circle similar in general form to those of terminal circle, except that root-processes are shorter and less easily observed. Basal hooks without recurved roots. Parasitic in fish.

Type species : *G. gracilisentis* (Van Cleave, 1913).

Genus (5) *Pandosentis* Van Cleave, 1920.

Diagnosis.—*Neoechinorhynchidae*, with the characters of the family, except for the variation in arrangement of giant nuclei within the sub-cuticle. These do not always lie in the sagittal plane, as in representatives of all the other genera previously included in this family, but are frequently lateral in distribution. Body proper small, devoid of spines. Proboscis short, cylindrical, provided with more than three circles of hooks. Boundary between root and thorn usually not sharply marked. Arrangement of male genital organs as in members of the genus *Gracilisentis*. Testes elliptical, contiguous. Prostatic gland a rounded syncytial mass immediately following the posterior testis, with its posterior boundary indented for the reception of the reservoir of the prostatic gland. Prostatic gland in the only known species contains sixteen giant nuclei. Central nervous system at base of proboscis-sheath. Retractors of sheath emerge from the sheath at its posterior extremity on dorsal and ventral surfaces. Lemnisci not as long as the proboscis-sheath. Parasitic in fish.

Type species : *P. iracundus* Van Cleave, 1920.

Family (2). QUADRIGYRIDAE Van Cleave, 1920.

Neoechinorhynchidea of medium size. Anterior body region provided with cuticular spines. Proboscis-sheath enclosed by a single muscular wall. Central nervous system located near the base of the proboscis-sheath. Subcuticular nuclei in anterior region elliptical, in sagittal plane; in remainder of body a few large, branched nuclei laterally arranged. Parasitic in fish.

The family contains only a single genus.

Genus *Quadrigyryus* Van Cleave, 1920.

Diagnosis.—Quadrigyridae of medium size. Proboscis armed with four circles of hooks. Anterior surface of body usually provided with four circles of cuticular spines. Subcuticular nuclei of two types; those of anterior part of body ovoid giant nuclei, dorsal and ventral in location; those in remainder of body a large, central elongated mass, from which heavy lateral projections are given off, usually lateral in distribution. Proboscis-sheath provided with a single, heavy muscular wall. Central nervous system located near posterior extremity of proboscis-sheath. Parasitic in fish.

Type species: *Q. torquatus* Van Cleave, 1920.

Family (3) APORORHYNCHIDAE Shipley, 1900.

Neoechinorhynchidea of short form with the body divided into three well-marked regions. The head (proboscis) is pitted but not armed with hooks. There is no eversible introvert, no proboscis-sheath and no armature of hooks. The sub-cuticle and lemnisci have a few giant nuclei, and the lemnisci are long and coiled. Parasitic in birds.

The family contains only a single genus.

Genus *Apororhynchus* Shipley, 1900.

SYNONYM:—*Arbynchus* Shipley, 1896.

With the characters of the family.

Type species: *A. hemignathi* Shipley, 1896.

With regard to this species, Marval (1905) writes: 'Nous nous permettrons donc, maintenant, de considérer *Arhynchus hemignathi*

comme un *Neorhynchus*, endoparasite comme tous les Acanthocéphales, sans exception, et privé de rostre soit accidentellement ce qui est probable, soit à la suite de longues modifications telles que celles qui se produisent chez l'*Echinorhynchus filicollis* et *sphaerocephalus*, lors de la transformation du rostre en bulle.'

Sub-order II. GIGANTORHYNCHIDEA

Proboscis reduced, often composing only a small part of the proboscis-like structure ; proboscis-sheath with a thick muscular wall into which the proboscis (when present) cannot be retracted, the proboscis-sheath being inserted near the anterior extremity. Neck present. Nuclei of the sub-cuticle and lemnisci relatively small and numerous. Prostatic glands not a single syncytial mass. Parasitic in mammals and birds.

The order is divided into two families.

KEY TO THE FAMILIES OF THE SUB-ORDER GIGANTORHYNCHIDEA.

- Proboscis greatly reduced, represented by only one or two transverse rows of large hooks at the anterior extremity of the proboscis-like structure. Neck armed with numerous small hooks..... *Gigantorhynchidae* (1)
- Proboscis sub-spherical, armed with 5 or 6 transverse rows of hooks. Neck unarmed..... *Oligacanthorhynchidae* (2)

Family (1) GIGANTORHYNCHIDAE Hamann, 1892.

Gigantorhynchidea of large size. Body apparently segmented. Proboscis rudimentary, represented by one or two transverse rows of hooks. Hooks with double roots. Neck armed with numerous small hooks. Lemnisci filiform, with numerous nuclei. Testes ellipsoidal, elongated, situated posteriorly. Prostatic glands sub-spherical. Parasitic in mammals.

The family contains only a single genus.

Genus *Gigantorhynchus* Hamann, 1892.

SYNONYM :—*Echinorhynchus* Zoega, 1776, in part.

With the characters of the family.

Type species : *G. echinodiscus* (Diesing, 1851).

Family (2) OLIGACANTHORHYNCHIDAE, nom. nov.

Gigantorhynchiea of small to large size. Body more or less rugose. Proboscis sub-spherical or nail-like, armed with five or six transverse rows of hooks. Hooks (excepting those at the base) with double roots. Neck short, unarmed. Testes ellipsoidal or cylindrical. Prostatic glands eight, ellipsoidal or nail-like. Parasitic in mammals and birds.

The family contains three genera.

KEY TO THE GENERA OF THE FAMILY OLIGACANTHORHYNCHIDAE.

1. Sexual dimorphism well marked; females very large and spirally coiled, males small, comma-shaped. Lemnisci relatively short and flat. Testes situated some distance anterior to the prostatic glands. Genital organs of the male occupying two-thirds of the body cavity..... *Macracanthorhynchus* (1)
Sexual dimorphism not well-marked. Lemnisci narrow and cylindrical..... 2
2. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity..... *Oligacanthorhynchus* (2)
Genital organs of the male occupying two-thirds or more of the body cavity..... *Prosthenorchis* (3)

Genus (1) *Macracanthorhynchus* Travassos, 1917.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.

Gigantorhynchus Hamann, 1892, in part.

Diagnosis.—Sexual dimorphism well-marked; females very large and spirally coiled, males small, comma-shaped. Proboscis very large. Lemnisci rather short and flat, extending backwards to the anterior testis. Genital organs of the male occupying two-thirds of the body cavity. Testes long, cylindrical. Parasitic in mammals.

Type species: *M. hirudinaceus* (Pallas, 1781).

A single species belonging to this genus was found in the collection, namely :—

Macracanthorhynchus hirudinaceus (Pallas, 1781).

SYNONYMS :—*Taenia haeruca* Pallas, 1766, preoccupied, in part.

Taenia hirudinacea Pallas, 1781.

Echinorhynchus gigas Bloch, 1782.

Gigantorhynchus gigas of Hamann, 1892.

Gigantorhynchus birudinaceus of Porta, 1908.

Six females and five males; host unknown. Hong Kong, January, 1914 (Dr. Bell). Also one male and one female; host unknown. Kindly lent by A. W. Noel Pillers, F.R.C.V.S., D.V.S.M.

The largest female measured 532 mm. in length, and 9 mm. in greatest breadth. The largest male measured 80 mm. in length, and 6 mm. in greatest breadth.

Genus (2) *Oligacanthorhynchus* Travassos, 1915.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hamania Travassos, 1915.
Hamanniella Travassos, 1915.

Diagnosis.—Sexual dimorphism not well-marked. Lemnisci filiform or cylindrical, long, with numerous nuclei. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity. Testes ellipsoidal. Parasitic in mammals (marsupials and edentates) and birds.

Type species : *O. spira* (Diesing, 1851).

In place of the genus *Oligacanthorhynchus*, Travassos recognises two genera, namely, *Oligacanthorhynchus* and *Hamanniella*, which are very closely allied but, according to Travassos, may be distinguished as follows :—

Prostatic glands ellipsoidal, in pairs. Parasitic in birds..... *Oligacanthorhynchus*
 Prostatic glands nail-like, condensed. Parasitic in marsupials
 and edentates..... *Hamanniella*

The distinction based on the shape of the prostatic glands appears to us to be difficult to make out, and is not clearly shown in at any rate one of Travassos' figures, and therefore we have included both genera in a single genus for which the name *Oligacanthorhynchus* appears to have priority.

A single species belonging to this genus was found in the collection, namely :—

Oligacanthorhynchus microcephalus (Rud., 1819).

SYNONYMS :—*Echinorhynchus microcephalus* Rud., 1819.
Hamania microcephala Travassos, 1915.
Hamanniella microcephala Travassos, 1915.

One male specimen from the intestine of *Didelphis marsupialis*. British Guiana, 1912 (Dr. Minett).

Unfortunately the proboscis is missing, and consequently a definite identification is not possible. The incomplete worm measured about 35 mm. in length. Our specimen agrees in general with Travassos' figure of the male of this species, excepting that the lemnisci are, relatively, extremely long (about 17 mm.) and extend to the testes. Our specimen is young and consequently much shorter than the fully-developed specimen figured by Travassos, the length of which is given as 150 mm. to 200 mm. ; this fact probably accounts for the apparent difference in the length of the lemnisci in the two specimens.

Genus (3) *Prosthenorchis* Travassos, 1915.

SYNONYMS :—*Oncicola* Travassos, 1916.

Pardalis Travassos, 1917, preoccupied.

Echinopardalis Travassos, 1918.

Diagnosis.—Oligacanthorhynchidae of small to medium size. Sexual dimorphism not well-marked. Body rugose. Proboscis sub-spherical, armed with five or six transverse rows of hooks. Testes situated in the middle third of the body or more anteriorly ; genital organs of the male occupying two-thirds or more of the body cavity. Ejaculatory canal very long. Parasitic in mammals and birds.

Type species : *P. spirula* (Olfers, in Rudolphi, 1819).

In place of the genus *Prosthenorchis*, Travassos recognises three genera, namely *Oncicola*, *Echinopardalis*, and *Prosthenorchis*, which are very closely allied but, according to Travassos, may be distinguished as follows :—

1. Testes small, round. Prostatic glands large, condensed, situated just behind the testes..... *Oncicola*
 Testes larger, ellipsoidal. Prostatic glands not unusually large..... 2
2. Prostatic glands ovoid, in pairs..... *Echinopardalis*
 Prostatic glands ellipsoidal, not in pairs..... *Prosthenorchis*

Some of Travassos' figures, however, do not fully support these distinctions, and therefore we have included all three in a single genus for which the name *Prosthenorchis* has priority.

Two species belonging to this genus were found in the collection, namely :—

Prosthenorchis spirula (Olfers, in Rud., 1819).

SYNONYMS :—*Echinorhynchus spirula* Olfers, in Rud., 1819.
Echinorhynchus elegans Diesing, 1851.
Prosthenorchis elegans Travassos, 1915.

Nine specimens, including two males, from the intestine of monkeys ; species and locality unknown.

In Travassos' figure of the male of *P. elegans*, the worm is shown to be short and broad, the lemnisci overlap the anterior testis, the two testes strongly overlap, and the prostatic glands are compacted into a single oval mass immediately behind them. In his figure of *P. spirula*, the entire worm is shown elongated, the lemnisci, although long, extend only half-way to the anterior testis, the two testes do not overlap but are situated one behind the other in the middle third of the body, and the prostatic glands are placed single file one behind the other, forming a long cylindrical mass.

Our male specimens show characters intermediate between the two above species ; thus, the body is relatively long, the lemnisci overlap the anterior testis, the testes are slightly separated, lying one in front of the other, and the prostatic glands in one male form a compact mass as in *P. elegans*, but in the other, the anterior glands are drawn out as in *P. spirula*, whilst the posterior glands are compacted as in *P. elegans*.

For the above reasons we consider *P. elegans* is indistinguishable from *P. spirula*.

It may be noted that in our specimens the eggs were similar in shape and size to those figured by Travassos for both the above species, and that the average measurements of ten eggs were 78μ by 47μ .

Prosthenorchis pardalis (Westrumb, 1821).

SYNONYMS :—*Echinorhynchus pardalis* Westrumb, 1821.
Echinorhynchus ovatus Leidy, 1850.
Echinorhynchus campanulatus Diesing, 1851.
Echinorhynchus oncicola v. Ihering, 1902.
Oncicola oncicola Travassos, 1916.
Pardalis pardalis Travassos, 1917.
Echinopardalis pardalis Travassos, 1918.

Numerous specimens, males and females, from the intestine of *Felis pardus*. Freetown, Sierra Leone, 10.III.1923 (Professor B. Blacklock and Dr. S. Adler).

Size. The females measured from 7 mm. to 14 mm. in length, and from 1.2 mm. to 1.8 mm. in breadth; only one of the females, viz., the longest, was gravid. The males varied in length from 8 mm. to 15 mm., and in breadth from 1.3 mm. to 1.8 mm.; only the larger males were mature. Travassos states that *Echinopardalis pardalis* has the following measurements: female, 30 mm. to 40 mm. by 1 mm. to 2.5 mm.; male, 30 mm. by 1 mm. to 1.5 mm. Our specimens are therefore much smaller than Travassos' specimens of *E. pardalis* and correspond more closely in length to his *Oncicola onicola* which measures as follows:—female, 10 mm. to 13 mm. by 3 mm. to 4 mm.; male, 9 mm. to 11 mm. by 2.5 mm. to 3 mm.

Diesing's specimens of *E. campanulatus* measured 6 mm. to 35 mm. in length, and from 2 mm. to 6 mm. in breadth.

Shape of body. Our specimens varied within fairly wide limits; all were slightly curved, some being cylindrical and tapering at each end, whilst others were more club-shaped, the broad end being anterior. The latter included specimens which were obviously shrunk. The skin, in the majority of the specimens, was smooth and ringed, but in others it was definitely wrinkled or rugose. Our specimens possess a peculiar collar-like structure identical with that figured by Diesing for his *E. campanulatus*. Travassos states that one of the differences between *O. onicola* and *E. pardalis* is that the former possesses a 'neck' and the latter does not; but at the same time he gives Diesing's *E. campanulatus* as a synonym of *E. pardalis*.

Proboscis-sheath. The muscular wall of the proboscis-sheath is very thick and, when viewed in certain positions, resembles the letter 'J.' The central nervous system is situated eccentrically, slightly posterior to the middle, and close to the end of the short limb of the muscular 'J.' The anterior ends of the muscular portion of the proboscis-sheath are connected with the proboscis by non-muscular strands.

Lemnisci. The lemnisci are very long, extending to the posterior third of the body, and often reaching nearly to the posterior extremity. In this character the specimens resemble *O. onicola*.

Testes. These lie near the middle of the body excepting in one or two specimens in which they are situated immediately behind the proboscis-sheath. The relative position of the testes is perhaps

to some extent dependent, firstly on the body contraction, and secondly on the contraction of the muscles attached to the proboscis-sheath, which tends to move the sheath posteriorly. The testes lie one in front of the other and are about twice as long as broad; the largest testis measured 0.97 mm. by 0.46 mm. In *E. pardalis* the testes measure 2 mm. to 3 mm. in length by 0.5 mm. in breadth, whilst in *O. onicicola* they measure 0.8 mm. to 1 mm. in diameter.

The testes in our specimens thus resemble those of *O. onicicola* in length, they are intermediate between those of *E. pardalis* and *O. onicicola* in shape and appearance, whilst they resemble those of both species as regards their position.

Eggs. The eggs in our specimens averaged about 65μ by 45μ . Travassos gives the size of the egg of *E. pardalis* as 53μ to 63μ by 38μ to 42μ , and that of *O. onicicola* as 99μ by 71μ to 75μ . The eggs of our specimens thus resemble more closely those of *E. pardalis*.

It will be clear then that our specimens resemble *Oncicola onicicola* in some characters and *Echinopardalis pardalis* in others, and the facts cited above lead us to the conclusion that the two forms are identical, *Oncicola onicicola* being merely the contracted form of *Echinopardalis pardalis*.

Sub-order III. ECHINORHYNCHIDEA

Proboscis well-developed; proboscis-sheath with double walls (except in the genus *Mediorhynchus*) into which the proboscis can be retracted. Nuclei of the sub-cuticle and lemnisci relatively small and numerous, or with few large, finely dendritic nuclei. Prostatic glands not a single syncytial mass.

The order is divided into four families.

KEY TO THE FAMILIES OF THE SUB-ORDER ECHINORHYNCHIDEA.

1. Proboscis long, armed with numerous hooks which are stronger on the ventral than on the dorsal aspect..... *Rhadinorhynchidae* (1)
 Proboscis armed with hooks arranged radially and symmetrically.....2
2. Proboscis sheath inserted near the middle of the proboscis-like structure, that is, the neck is armed with spines..... *Centrorhynchidae* (2)
 Proboscis sheath inserted at the base of the proboscis; neck absent or unarmed.....3
3. Anterior region of body, in males at least, clothed with cuticular spines..... *Corynosomidae* (3)
 Anterior region of body without spines.....4
4. Body moniliform..... *Moniliformidae* (4)
 Body not moniliform..... *Echinorhynchidae* (5)

Family (I) RHADINORHYNCHIDAE Travassos, 1923.

Echinorhynchidea of small to medium size. The anterior region of the body armed with scattered cuticular spines (except in the genus *Leptorhynchoides*). Proboscis long (at least twice as long as broad, and often much longer), usually bent ventrally, and armed with numerous hooks which are stronger on the ventral than on the dorsal aspect. Basal portion of proboscis often without hooks. Neck absent. Proboscis-sheath long. Central nervous system near the middle of the proboscis-sheath. Eggs with or without polar capsules. Parasitic in fish, reptiles and birds.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY RHADINORHYNCHIDAE.

1. Body not armed with spines..... *Leptorhynchoides* (2)
Body armed with spines.....2
 2. Body with ventral transverse rows of spines..... *Serrasentis* (4)
Body without ventral transverse rows of spines.....3
 3. Posterior extremity of the body in both sexes armed with
a few scattered cuticular spines..... *Telosentis* (5)
Posterior extremity unarmed.....4
 4. Body covered anteriorly with scattered, powerful spines ;
not differentiated into two structurally distinct
portions. Proboscis sub-cylindrical, hooks on dorsal
and ventral aspects not differing notably in size, but
more in a varied formation of their roots..... *Rhadinorhynchus* (1)
- Body with anterior region sharply differentiated
structurally. Proboscis spindle-shaped, hooks on
dorsal and ventral aspects differing distinctly in size..... *Arhythmorhynchus* (3)

Genus (I). *Rhadinorhynchus* Lühe, 1911.

SYNONYMS :—*Polyacanthorhynchus* Travassos, 1918.

Echinosome Porta, 1907, preoccupied, in part.

Diagnosis.—Rhadinorhynchidae with very long, cylindrical proboscis, and very long lemnisci. Anterior portion of body not structurally differentiated from the rest. Body armed at the anterior end with large, scattered, cuticular spines, but without ventral transverse rows of body spines. Parasitic in fish and reptiles.

Type species : *R. pristis* (Rudolphi, 1802).

A single species belonging to this genus was found in the collection, namely :—

Rhadinorhynchus pristis (Rudolphi, 1802).

SYNONYM :—*Echinorhynchus pristis* Rudolphi, 1802.

Four males from the intestine of *Thynnus vulgaris*. Locality unknown.

Genus (2). *Leptorhynchoides* Kostylev, 1924.

Diagnosis.—Rhadinorhynchidae with very long, slightly club-shaped proboscis, and very long lemnisci. Body not armed with spines; nuclei dendritic. Parasitic in fish.

Type species: *L. plagicephalus* (Westrumb, 1821).

Genus (3). *Arhythmorhynchus* Lühe, 1911.

Diagnosis.—Rhadinorhynchidae with a long spindle-shaped proboscis; without ventral transverse rows of body spines. Lemnisci slightly longer than proboscis-sheath. Anterior region of body sharply differentiated from posterior region in structure of body wall; nuclei present in the sub-cuticle of anterior region only. Parasitic in birds.

Type species: *A. frassoni* (Molin, 1858).

Genus (4). *Serrasentis* Van Cleave, 1923.

SYNONYMS :—*Echinogaster* Monticelli, 1905, preoccupied.

Echinosome Porta, 1907, preoccupied.

Lepidosoma Porta, 1907, preoccupied.

Diagnosis.—Rhadinorhynchidae with ventral transverse rows of body spines. Lemnisci very long. Parasitic in fish.

Type species: *S. socialis* (Leidy, 1851).

A single species belonging to this genus was found in the collection, namely :—

Serrasentis socialis (Leidy, 1851).

SYNONYMS :—*Echinorhynchus socialis* Leidy, 1851, not Leidy, 1856.

Echinorhynchus sagittifer Linton, 1889.

Echinogaster (species not stated) Monticelli, 1905.

Echinosome sagittifer of Porta, 1907.

Echinogaster sagittifer of Lühe, 1912.

Thirty-five specimens found encysted in the body cavity of *Platycephalus fuscus* ('Flathead'). Townsville, Queensland, Australia, 12.1.1921 (Dr. P. A. Maplestone).

All the specimens were adult but immature; they varied in length from about 3 mm. to 8 mm., and the maximum breadth was about 0.6 mm. The specimens agreed in general with Linton's description of *E. sagittifer*, but the following points of difference were noted:—(1) the number of hooks on the proboscis, counted antero-posteriorly, varied from about sixteen to eighteen, and there were about twenty-four such rows; (2) the number of ventral transverse rows of body spines varied from about fourteen to sixteen. There is no neck. The lemnisci arise at the base of the proboscis, and are very long, extending a little beyond the middle of the body. Central nervous system situated about the middle of the proboscis-sheath.

Van Cleave (1918) re-described the species and later (1923) erected the genus. In his description he states that the number of spines in the ventral transverse rows varied from six to twenty-four; this presumably means on each side as stated by Linton. In our specimens the first row contained about forty-five, the number decreasing in posterior rows.

Genus (5). *Telosentis* Van Cleave, 1923.

Diagnosis.—Rhadinorhynchidae with the posterior extremity of the body adjacent to the genital orifice armed in both sexes with a few scattered cuticular spines. Genital orifice sub-terminal. Parasitic in fish.

Type species: *T. molini* Van Cleave, 1923.

Family (2). CENTRORHYNCHIDAE Van Cleave, 1916.

Echinorhynchidea of small to medium size. Proboscis-sheath inserted near the middle of the proboscis-like structure; that is to say, the neck is armed with spines. Hooks on the proboscis distinct in type from, and usually larger than, those on the neck. Central nervous system situated near the middle of the proboscis-sheath. Eggs where known without polar capsules. Parasitic in birds.

The family contains three genera.

KEY TO THE GENERA OF THE FAMILY CENTRORHYNCHIDAE.

1. With three prostatic glands..... *Centrorhynchus* (1)
 With eight prostatic glands.....2
2. Proboscis-sheath with a single wall..... *Mediorhynchus* (2)
 Proboscis-sheath with a double wall..... *Empodius* (3)

Van Cleave (1924) states 'that the names *Heteroplus* and *Mediorhynchus* have been applied to the identical generic concept,' and that, moreover, the generic name *Empodius* is a synonym of *Mediorhynchus*, and has been recognised as such by its author Travassos. As the prior name *Heteroplus* is preoccupied, the valid name for the genus becomes *Mediorhynchus*.

In suggesting this synonymy, Van Cleave has apparently disregarded one of the characteristics of his genus *Mediorhynchus*, namely, that 'the wall of the proboscis receptacle is composed of a single muscular layer instead of two layers' (a feature which is well shown in his figure accompanying his description of the type species *M. papillosus*), for in the genera *Heteroplus* and *Empodius* the proboscis-sheath has a double wall. Again, on comparing the figures given by Van Cleave of the proboscis-like structure of *M. papillosus*, the type species of the genus *Mediorhynchus*, and of *M. grandis*, which was subsequently placed by him in the genus *Heteroplus*, there is seen to be an important difference, the number of longitudinal rows of hooks on the neck being in *M. papillosus* about the same as on the proboscis proper (in this respect resembling species of the genus *Centrorhynchus*), whereas in *M. grandis* they are much more numerous.

Having regard to these two important differences we are unable to accept without further explanation Van Cleave's suggested synonymy, and we therefore recognise in this paper two genera in place of his *Mediorhynchus*.

With regard to the genus *Micracanthorhynchus* Travassos, 1917, Van Cleave maintains that it is a synonym of his *Mediorhynchus*†. He bases this conclusion on a re-examination of Rudolphi's type of *E. micracanthus*, a species which Travassos states is closely related to *M. emberizae*, the type species of the genus *Micracanthorhynchus*. Van Cleave has figured the anterior extremity of *E. micracanthus*, and from this figure it appears probable that the species should be referred to the genus *Empodius*.*

Genus (1). *Centrorhynchus* Lühe, 1911.

SYNONYMS :—*Paradoxites* Lindemann, 1865.

Chentrosoma Monticelli, 1905, in part.

Diagnosis.—*Centrorhynchidae* having a proboscis-sheath with double walls. Proboscis and neck bearing approximately equal

* See Addendum to this paper, p. 182.

numbers of longitudinal rows of hooks. Prostatic glands three (Van Cleave), long and tubular.

Type species: *C. aluconis* (Müller, 1780 or 1784).

A single species belonging to this genus was found in the collection, namely :—

Centrorhynchus asturinus (Johnston, 1913).

SYNONYM :—*Gigantorhynchus asturinus* Johnston, 1913.

One male and one female from the intestine of the sparrow-hawk (*Accipiter cirrocephalus*). Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone).

The male measured 18 mm. in length, and the maximum breadth was 0.6 mm. The female measured 25 mm. in length, and the maximum breadth was 0.8 mm. The body is slightly curved and cylindrical; in both specimens there was a small constriction which, in the male, was situated immediately behind the testes, and in the female a little way behind the ends of the lemnisci. The cuticle is smooth. The proboscis-like structure measures 0.85 mm. by 0.25 mm.; it is armed with numerous hooks radially arranged in about forty antero-posterior rows of about thirty hooks each. The hooks on that portion of the proboscis anterior to the insertion of the sheath are larger than the rest, and have long rectangular roots. The neck is marked off from the commencement of the body proper by a slight constriction.

Proboscis-sheath. The sheath arises a little anterior to the middle of the proboscis-like structure; it measures about 1.3 mm. in length, and the maximum breadth is about 0.25 mm. The central nervous system lies a little posterior to the middle of the sheath.

Lemnisci. These organs extend backwards to a level a little posterior to the proboscis-sheath.

Testes. The testes are oval, lie one in front of the other, and are in apposition. They lie immediately behind the proboscis-sheath. Each testis measures about 0.9 mm. in length and 0.28 mm. in breadth.

Prostatic glands. These commence immediately behind the testes and are cylindrical and extremely long.

Female. The posterior extremity of the female is produced into a short, blunt, conical protuberance.

Eggs. These measure about 55μ by 22μ , and are without polar capsules.

Johnston's original description (1913) was based on the examination of a few specimens from *Astur novae-hollandiae* obtained in the neighbourhood of Townsville. He pointed out that the specimens were very much coiled, and we presume he had difficulty in examining them fully because he subsequently published an emended description (1918).

In addition to the two well-preserved specimens described above, we have at our disposal a few other specimens of this species from the same locality, obtained from the intestine of a white goshawk (*Astur novae-hollandiae*). These specimens were very much coiled, as were Johnston's. An examination of these coiled specimens showed clearly that they were morphologically identical with the specimens from *Accipiter cirrocephalus*, but were a little longer and narrower.

We have also examined three male and seven female specimens of the same species from the intestine of a grey goshawk (*Astur clarus*) obtained in the neighbourhood of Townsville, 3.6.1912 (Dr. P. A. Maplestone). The largest female specimen measures 60 mm. in length and 1 mm. in breadth. The terminal papilla noted above is absent, the specimen being distended with eggs.

Also one male and two females, all immature, from the intestine of a brown hawk (*Hieracidea orientalis*) obtained in the neighbourhood of Townsville, 19.6.1913 (Dr. P. A. Maplestone). The only point in these specimens calling for comment is the relative position of the testes which are situated a little in front of the middle of the worm. The prostatic glands are rudimentary. These differences are probably due to the worm being immature.

There seems to be little doubt but that all the forms examined by us are specimens of *Centrorhynchus asturinus* (Johnston, 1913). If this surmise is correct, then Johnston's description can be somewhat amplified by details observed in the better preserved specimens, especially with regard to the number and character of the hooks on the proboscis.

Genus (2). *Mediorhynchus* Van Cleave, 1916.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a single wall. Longitudinal rows of hooks on the proboscis and neck similar in number. Prostatic glands eight, rounded or pear-shaped.

Type species : *M. papillosus* Van Cleave, 1916.

Genus (3). *Empodius* Travassos, 1916.

SYNONYMS :—*Heteroplus* Kostylev, 1914, preoccupied.
Micracanthorhynchus Travassos, 1917.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a double wall. Proboscis and neck bearing different numbers of longitudinal rows of hooks, those on the neck being the more numerous. Prostatic glands eight, rounded or pear-shaped.

Type species : *E. empodius* Skrjabin, 1913.

A single species belonging to this genus was found in the collection, namely :—

Empodius segmentatus (Marval, 1902).

Four females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Transvaal, 1907 (G. Arnold). Also three males and five females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Upper Shire, Nyasaland, 1911 (Professor R. Newstead and Dr. Davey).

The males measured from 62 mm. to 74 mm. in length, and the greatest breadth was 2.3 mm. ; the number of pseudo-segments varied from fifty-eight to seventy-three. The females measured from 65 mm. to 90 mm. in length, and the greatest breadth was 2.3 mm. ; the number of pseudo-segments varied from sixty-three to eighty-eight. The body is tape-like and flattened laterally, and the pseudo-segments extend practically to both extremities ; the body is broadest anteriorly and tapers gradually and continuously towards the posterior extremity. In the female the posterior extremity is bluntly rounded, but in the male, when the bursa is retracted, there are at the posterior extremity two conspicuous lateral folds.

Proboscis. In the majority of our specimens the proboscis is retracted and the anterior extremity of the worm is quite rounded.

In some specimens the proboscis lies slightly ventrally, whilst in others it is median. When the proboscis is completely protruded it is continuous with the anterior part of the body, from which it can only be distinguished by the presence of large hooks. In this condition it is evident that two distinct types of hooks are present on the anterior part of the worm, namely, a few large hooks situated anteriorly, on the proboscis, and a large number of small hooks situated more posteriorly, on the neck. When the proboscis is retracted, as it is in most of our specimens, the cuticle at the anterior extremity is invaginated and consequently the small hooks are more or less hidden, the number visible depending on the degree of retraction.

The proboscis is small and bluntly conical ; it measures about 0.25 mm. in length, and its diameter across the base is about 0.4 mm. It is armed with about twenty antero-posterior rows each composed of four hooks ; the hooks measure about 45μ to 55μ , and have large root-like processes. On the neck are at least forty antero-posterior rows each composed of about four hooks ; the hooks are very delicate, slender, and decrease in size posteriorly, the anterior hooks measuring about 26μ to 40μ in length. These small hooks have no root-like processes.

Proboscis-sheath. The proboscis-sheath has double walls and arises at the base of the proboscis proper ; it is slightly curved and tapers a little posteriorly. It measures about 1.2 mm. in length, and its greatest breadth is 0.4 mm. The central nervous system is situated about the middle of the sheath.

Lemnisci. These measure about 3 mm. to 4 mm. by 0.3 mm.

Testes. The position of the testes varies slightly, but in all our specimens they lie in the posterior quarter of the worm. They are separated from each other by a short interval. Each testis is an elongated oval body measuring from 2.7 mm. to 3.8 mm. in length and in greatest breadth from 0.9 to 1.1 mm.

Prostatic glands. These lie a little distance behind the testes, and consist of eight more or less elongated bodies, loosely compacted together. In one male they extended over 7 mm. of the body length, but in another over only 3.9 mm.

Eggs. The average size of ten eggs was 87μ by 50μ .

In 1902, Marval described a worm from *Numida ptilorhynchus*

to which he gave the name *Echinorhynchus segmentatus*. Of this worm he had only a single specimen, the sex of which was not determined, and the proboscis of which was missing. His description, therefore, was necessarily incomplete, but considering the facts that the worm came from the same host as our specimens, that its body was divided into a similar number of pseudo-segments, and that the eggs were alike, we have little hesitation in concluding that it was probably of the same species as our specimens, and accordingly we have adopted Marval's specific name. From our more abundant and complete specimens we have been able to supplement Marval's earlier description.

Family (3). CORYNOSOMIDAE nom. nov.

Echinorhynchidea of small to rather large size. Anterior region of the body in the males, and (except perhaps in some species of *Filicollis*) in the females also, clothed with closely-set cuticular spines which extend backwards as a mantle for a variable distance. Proboscis armed with hooks arranged radially and symmetrically, i.e., without any distinction in size between those situated dorsally and those situated ventrally. Neck, when present, without spines. Eggs either with or without polar capsules. Parasitic in cetacea, birds and fish.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY CORYNOSOMIDAE.

1. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at anterior end of proboscis-sheath..... *Tegorhynchus* (5)
 Proboscis not covered by such a membrane. Central nervous system at, or posterior to, middle of proboscis-sheath.....2
2. Body proper dilated into a bulb anteriorly..... *Bolbosoma* (2)
 Body proper not so dilated.....3
3. Proboscis bent ventrally at an angle with the axis of the body. Spines on the anterior part of the body extending backwards much further ventrally than dorsally..... *Corynosoma* (1)
 Proboscis not bent ventrally. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally.....4
4. Body sac-like, not notably thickened anteriorly. Prostatic glands irregularly egg-shaped..... *Filicollis* (4)
 Anterior region of body thickened. Prostatic glands tubular... *Polymorphus* (3)

Genus (1). *Corynosoma* Lühe, 1904.

Diagnosis.—Corynosomidae of small to medium size. Body club-shaped, the anterior end thickened but not separated from the posterior part. Spines on anterior part of body extending much further backwards ventrally than dorsally. Genital opening in the male armed with hooks. Proboscis bent ventrally, often spindle-shaped and unarmed at the base. Central nervous system near the middle of the proboscis-sheath. Lemnisci short. Eggs with polar capsules. Parasitic in birds.

Type species: *C. strumosum* (Rudolphi, 1802).

Genus (2). *Bolbosoma* Porta, 1908.

SYNONYM :—*Bolborbynchus* Porta, 1906, preoccupied.

Diagnosis.—Corynosomidae of rather large size. Body properly dilated anteriorly, a little behind the proboscis, into a bulb. Spines on the anterior part of the body not extending posterior to the dilation. Proboscis short, sub-cylindrical, unarmed at its base. Neck absent. Central nervous system near the middle of the proboscis-sheath. Eggs long and narrow, with polar capsules. Parasitic in cetacea.

Type species: *B. capitatus* (v. Linstow, 1880).

Genus (3). *Polymorphus* Lühe, 1911.

Diagnosis.—Corynosomidae of small size. Body thickened anteriorly, sometimes narrowed immediately behind the spine-bearing region. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally. Genital opening in the male unarmed. Proboscis sub-cylindrical, often unarmed at the base. Central nervous system in the posterior third of the proboscis-sheath. Lemnisci of moderate length. Prostatic glands tubular. Eggs spindle-shaped, with polar capsules. Parasitic in birds.

Type species: *P. minutus* (Zed., 1800).

A single species belonging to this genus was found in the collection, namely :—

Polymorphus minutus (Zed., 1800).

Four females from the intestine of *Anas* sp., Egypt.

The specimens measured about 4 mm. to 6 mm. in length, and the greatest breadth was 1.3 mm.

The body is short and broad, tapering towards the posterior extremity, which is bluntly rounded. In one female the diameter of the anterior part was 1.3 mm., and that of the posterior part was 0.66 mm. Van Cleave states that in the genus *Polymorphus* the 'anterior end of the body is swollen and separated from the more attenuated posterior region by a constriction.' In our specimens this constriction, although clearly present in one specimen, was not obvious in the others, but neither is it shown in Van Cleave's figure of the male *P. obtusus*. The cuticle is smooth, but at the extreme anterior end it is closely beset with minute spines about $18\ \mu$ long; those on the ventral surface extend rather further back than those on the dorsal surface, but they do not extend beyond the anterior fifth of the worm, whilst the dorsal spines cover only about 0.4 mm. of the anterior dorsal surface.

Proboscis. The proboscis is situated somewhat ventrally and although bent slightly ventrally it is almost in line with the body. It is sub-cylindrical, slightly narrowed both anteriorly and posteriorly, and unarmed at the base. There is no neck. The length of the proboscis is about 0.5 to 0.6 mm., and its greatest breadth 0.3 mm. The proboscis is armed with numerous large hooks radially arranged and distributed in about sixteen antero-posterior rows each composed of about nine or ten hooks. The largest hooks are situated in the middle of the proboscis, measure about $70\ \mu$ in length, and have long rectangular root-like processes.

Proboscis-sheath. The sheath is curved in the form of an arc; it measures about 1.3 mm. in length and its greatest breadth is about 0.2 mm. The central nervous system lies a little posterior to the middle of the proboscis-sheath.

Lemnisci. The lemnisci are slightly longer than the proboscis-sheath, and arise at the base of the proboscis.

Eggs. The eggs in the body cavity are long and narrow and vary in size and appearance according to the degree of maturity. The fully mature egg measured about $120\ \mu$ by $30\ \mu$ (average of 10) and polar capsules were not seen. The embryo within it is cylindrical,

about $60\ \mu$ by $17\ \mu$, with rounded ends ; it is of a light brown colour and its surface presents a pitted or shagreen appearance. The eggs, immediately before becoming mature, show one or two polar capsules at each end, and the contained embryo is transparent. Some eggs thus resemble Lühe's figure of the egg of *P. minutus*, whilst others resemble Marval's figure of the egg of *E. anatis*.

Genus (4). *Filicollis* Lühe, 1911.

Diagnosis.—Corynosomidae of medium size. Body sac-like, anterior part not notably thickened, armed with spines anteriorly which do not extend much further backwards ventrally than dorsally. In the male the spines are well-developed, but in the gravid female they may be very small and well-nigh unrecognisable. Genital opening unarmed. Proboscis spherical or ovate ; in the female the proboscis may be bulbular and bear hooks only on its anterior extremity. Neck long and unarmed. Central nervous system in the posterior third of the proboscis-sheath. Prostatic glands irregularly egg-shaped. Eggs with or without polar capsules. Parasitic in birds.

Type species : *F. anatis* (Schrank, 1788).

Genus (5). *Tegorhynchus* Van Cleave, 1920.

Diagnosis.—Corynosomidae of small size. Posterior extremity of body unarmed ; in the female, terminating in two short, blunt papillae. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at the anterior extremity of the proboscis-sheath. Lemnisci long, about half the length of the body. Prostatic glands elongated. Parasitic in fish.

Type species : *T. brevis* Van Cleave, 1920.

Family (4). MONILIFORMIDAE Van Cleave, 1924.

Echinorhynchidea of medium to large size. Body without spines, and divided into a large number of pseudo-segments. Neck absent. Proboscis well developed, sub-cylindrical, armed with numerous hooks which are small and have only a single, posteriorly directed root. Lemnisci filiform, long, with numerous nuclei. Testes ellip-

soidal, situated quite posteriorly. Prostatic glands eight, almost spherical, compressed. Parasitic in rodents and insectivores.

The family contains only a single genus.

Genus *Moniliformis* Travassos, 1915.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hormorhynchus Ward, 1917.

With the characters of the family.

Type species : *M. moniliformis* (Bremser, 1811).

Two species belonging to this genus were found in the collection, namely :—

Moniliformis moniliformis (Bremser, 1811).

SYNONYMS :—*Echinorhynchus moniliformis* Bremser, 1811.
Gigantorhynchus moniliformis of Railliet *et al.*
Hormorhynchus moniliformis of Ward, 1917.
Echinorhynchus cestodiformis von Linstow, 1904.
Gigantorhynchus cestodiformis of Porta.
Moniliformis cestodiformis of Travassos.

A very large number of specimens were examined, from rats (*Rattus rattus* and *Rattus norvegicus*), collected in Liverpool, West Africa (Freetown and Accra), South America (Manaos), and Australia (Townsville). Also one specimen from man (British Honduras), and numerous specimens from *Cricetomys gambianus* (Accra). An examination of the above specimens led us to the conclusion that they were all of the same species and we give below a general account of its characters.

The males varied in length from 5.5 mm. to 86 mm., and the females from 7 mm. to 239 mm. In worms, from a single host, the size often varied within very wide limits, some being large and fully mature, whilst others were small and incompletely developed.

Shape. In very small and immature worms the body is sub-cylindrical and decidedly broader at the anterior extremity than it is posteriorly. In fully-developed worms, however, the body is flat, tape-like and, excepting at the two extremities, marked out into a large but variable number of pseudo-segments; the posterior end is somewhat broader than the anterior end.

Proboscis. Relatively short, cylindrical, with a broadly rounded end. Length, 0.5 mm. to 0.67 mm., greatest breadth about 0.2 mm.

Armed with twelve to sixteen (usually twelve) antero-posterior rows each composed of ten to twelve (usually eleven) hooks. The arrangement of the hooks is not quite regular. Hooks in the middle third of the proboscis about 25μ to 30μ in length; with a single root. The size, shape, and armature of the proboscis are similar in the worms regardless of their size. The proboscis was occasionally found retracted within the sheath, and frequently the entire proboscis was invaginated into the anterior extremity of the worm.

There is no neck, but the proximal end of the proboscis is devoid of hooks.

Proboscis-sheath. Large, with a double muscular wall, arising at the base of the proboscis. Length, 0.5 mm. to 1.3 mm.; greatest breadth, 0.22 mm. to 0.42 mm.

Lemnisci. Length, 2.4 mm. to 8.76 mm. Narrow, with a few large nuclei. Often very unequal. Lemnisci largest in the largest specimens.

Testes. Situated in the posterior part of the worm where they sometimes cause a slight swelling of the body; placed close together, the one anterior to the other. In the smallest immature worm examined by us they were sub-spherical and measured respectively 44μ and 63μ in diameter. In all the other specimens they were oval and usually elongated, in length varying from 201μ to 4 mm., and in greatest breadth from 120μ to 0.96 mm.; as a rule, in fully-developed worms they measured about 2 mm. to 2.5 mm. by 0.4 mm. In one specimen only a single testis was present.

Prostatic glands. Situated a little behind the posterior testis. There are, apparently, eight glands which are compacted together into a single oval mass and are usually individually indistinguishable. The mass of prostatic glands in mature worms varied in length from 0.45 mm. to 3.6 mm., and in greatest breadth from 0.25 mm. to 1.1 mm. In the smallest, immature specimens, the prostatic glands were almost unrecognisable.

Eggs. Rather variable in size and appearance. When fully mature the outer shell is slightly wrinkled and the enclosed embryo brown or dark-coloured. There are no polar capsules. In thirty eggs (ten from each of three females) measured by us, the length varied from 109μ to 137μ , average 123μ , and the greatest breadth from

57 μ to 63 μ , average 60.5 μ . Both larger and smaller eggs were, however, seen in other worms examined. It may be noted here that the smallest female examined by us in which there were eggs of the mature form (but not fully developed), measured only 13 mm. in length. In the same host we observed very much larger females (some 47 mm. long) which were without eggs.

The species is extremely variable in size, both in a single host and in different hosts. The proboscis is, however, remarkably constant in size. There seems to be no justification for dividing up the species on account of the variations in size which it exhibits.

Van Cleave (1924) states that he has examined von Linstow's type specimen of *M. cestodiformis* and that he has discovered no points of difference between this species and *M. moniliformis*. On account of the small size of the proboscis in *Hormorhynchus clarki* Ward, 1917, we are of opinion, however, that this species is probably distinct.

Moniliformis erinacei, sp.n.

One male and one female from the small intestine of a hedgehog (*Erinaceus europaeus*). Accra, Gold Coast, West Africa (Dr. J. W. S. Macfie).

The male measured about 85 mm. in length by 1.6 mm. in maximum breadth; the female measured about 110 mm. in length by 1.5 mm. in maximum breadth. The entire body, with the exception of the anterior and posterior extremities, is divided up into about 100 obvious pseudo-segments.

Proboscis. The proboscis measured about 0.4 to 0.5 mm. in length by about 0.2 mm. in maximum breadth. It is armed with numerous hooks arranged radially and distributed in eighteen antero-posterior rows each composed of seven to eight hooks, decreasing in size posteriorly. Each hook is short and stout, the largest measuring about 30 μ in length.

A pseudo-neck is present, which, when the proboscis is partly retracted, forms a prominent ruff or frill.

Proboscis-sheath. Its length is about 0.8 mm., and its greatest breadth 0.3 mm.

Lemnisci. The lemnisci are long, cylindrical, and relatively

narrow ; they measure 7 mm. to 8 mm. in length, and in greatest breadth 0.2 mm.

Testes. The testes are situated quite at the posterior extremity of the body ; they are large oval bodies measuring about 5 mm. in length and 1.3 mm. in breadth.

Prostatic glands. These form a somewhat compact mass immediately posterior to the testes.

Eggs. These resemble those figured by von Linstow, and measure (average of 10) 92μ by 51μ .

This worm agrees closely with von Linstow's description of *Echinorhynchus cestodiformis*, excepting that in the type specimens, which were from Nigerian hedgehogs, the lemnisci measured, in length, 1.7 mm. only, whilst in the specimens from the Gold Coast they measured from 7 mm. to 8 mm. Van Cleave (1924), however, has recently examined the type specimen of *M. cestodiformis* and has stated that it does not differ in any respect from *M. moniliformis*, and, therefore, the species described above, which differs from *M. moniliformis* in several respects, such as the size and armature of the proboscis and the dimensions of the eggs, must be regarded as a new species.

Family (5). ECHINORHYNCHIDAE Cobbold, 1879.

Echinorhynchidea of small to medium size. Body and neck (when present) without spines. Proboscis armed with hooks arranged radially and symmetrically. Eggs with or without polar capsules. Parasitic in mammals, birds, amphibians, and fish.

This family contains a heterogeneous group of species from which, during recent years, a number have been separated as distinct genera, leaving, however, a residue of species, not yet susceptible of more exact classification, in the original genus *Echinorhynchus*.

The genus *Plagiorhynchus* we regard as only another name for *Echinorhynchus*, the species referred to it appearing to be distinct only in the length of the lemnisci, and in that they occur in birds, characters which we do not consider to be of generic importance.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY ECHINORHYNCHIDAE.

1. With three prostatic glands..... *Prosthorhynchus* (1)
 With four prostatic glands..... *Oligoterorhynchus* (2)
 With six prostatic glands..... 2
2. Neck very long, expanded at its anterior extremity into a
 sub-spherical bulla..... *Pomphorhynchus* (3)
 Neck short or absent, without a bulla..... 3
3. Central nervous system at the posterior extremity of the
 proboscis-sheath..... *Acanthocephalus* (4)
 Central nervous system near the middle of the proboscis-
 sheath..... *Echinorhynchus* (5)

Genus (1). *Prosthorhynchus* Kostylev, 1916.

Diagnosis.—We have not been able to consult Kostylev's description of this genus, but according to Van Cleave (1923) the following appear to be its chief characteristics. Body without spines. Without giant nuclei. Proboscis very long, cylindrical or clavate, armed with hooks which are arranged radially and symmetrically. Neck short, unarmed. Proboscis-sheath sac-like, with double walls. Prostatic glands three, long and tubular. Parasitic in birds.

Type species : ?

Genus (2). *Oligoterorhynchus* Monticelli, 1914.

Diagnosis.—Echinorhynchidae of medium size. Proboscis sub-cylindrical, small, armed with numerous hooks. Base of proboscis unarmed. Neck absent. Lemnisci a little longer than the proboscis-sheath. Testes oval, situated in the middle third of the body. Prostatic glands four, long, sac-like, narrow. Parasitic in birds.

Type species : *O. campylurus* (Nitzsch, 1866).

Genus (3). *Pomphorhynchus* Monticelli, 1905.

Diagnosis.—Echinorhynchidae of small to medium size. Proboscis sub-cylindrical. Neck very long, cylindrical, excepting at its anterior extremity where in some species it expands into a sub-spherical bulla. Central nervous system at the posterior end of the proboscis-sheath. Parasitic in fish.

Type species : *P. laevis* (Zoega, 1776).

Genus (4). *Acanthocephalus* Koelreuter, 1771.

SYNONYM :—*Echinorhynchus* Zoega, 1776, in part.

Diagnosis.—Echinorhynchidae of small to large size. Proboscis short, ovate or cylindrical. Neck very short. Central nervous system at the posterior extremity of the proboscis-sheath. Parasitic in amphibians and fish.

Type species : *A. anguillae* (Müller, 1780).

A single species belonging to this genus was found in the collection, namely :—

Acanthocephalus bufonis (Shipley, 1903).

SYNONYM :—*Echinorhynchus bufonis* Shipley, 1903.

Three females and one male from the intestine of a toad. Hong Kong (Dr. Bell).

The male specimen measured 9 mm. in length, and the maximum breadth was 1.5 mm. ; the females measured from 20 mm. to 25 mm. in length, and the maximum breadth (near the anterior end) was 1.5 mm. to 1.8 mm.

Body cylindrical, slightly thickened anteriorly and tapering a little posteriorly, the posterior extremity being bluntly rounded. The body is curved, especially in the female, and the skin is smooth.

Proboscis. This organ is cylindrical and is situated asymmetrically as pointed out by Shipley ; length 0.5 mm. to 0.6 mm. ; breadth 0.3 mm. It is armed with eighteen to twenty antero-posterior rows each composed of six to eight hooks. The hooks are strongly geniculated at their base and in the middle of the proboscis measure 80μ to 90μ in length. The roots resemble those described by Lühe as present in *E. ranae*, i.e., they have no lateral wing-like expansions.

Proboscis-sheath. This measures about 1 mm. in length and 0.4 mm. in breadth, and is inserted at the base of the proboscis. Neck absent, or extremely short. The central nervous system lies at the posterior extremity of the proboscis-sheath.

Lemmisci. These are about twice as long as the proboscis-sheath and are rather broad.

Testes. These are situated at the beginning of the posterior half of the body ; they measure 0.6 mm. in length by 0.5 mm. in

breadth, and, in the single specimen examined, they lie one in front of the other and are in apposition.

Prostatic glands. These glands are elongated and extend to the posterior margin of the posterior testis.

Eggs. The eggs in the body cavity measured about 75μ by 26μ .

The specimens, therefore, agree with Shipley's description excepting as regards size. They differ from *E. ranae* in (1) the greater length of the lemnisci, and (2) the greater breadth of the eggs.

Genus (5). *Echinorhynchus* Zoega, 1776.

SYNONYM :—*Plagiorhynchus* Lühe, 1911.

Diagnosis :—Echinorhynchidae of small to large size. Proboscis long, sub-cylindrical, armed with numerous circles of alternating hooks. Hooks of almost uniform size excepting those of the few basal rows which are much reduced. Neck very short or absent. Central nervous system near the middle of the proboscis-sheath. Parasitic in mammals, birds, and fish.

Type species : *E. gadi* Zoega, 1776.

The following species found in the collection are referred to this genus :—

Echinorhynchus bazae, sp.n.

One male and two females from the intestine of a crested hawk (*Baza subcristata*). Townsville, Queensland, Northern Australia, 8.12.1913 (Dr. P. A. Maplestone).

The male measured 33 mm. in length, and the greatest breadth was 2 mm. Both females were incomplete, the fragments measuring 45 mm. and 50 mm. in length respectively, and about 2 mm. in breadth. Body rugose, without spines.

Proboscis. The proboscis is short and broad, slightly constricted about the middle, broadest in the basal half, with a rounded anterior extremity. In the male it measured 0.9 mm. by 0.64 mm., and in the females 1.2 mm. by 0.7 mm. The hooks, which extend to the base of the proboscis, are arranged radially in about thirty-eight to forty-one antero-posterior rows each composed of twelve or thirteen hooks. The hooks on the distal two-thirds are larger than the rest and have long rectangular root-like processes ; the larger (anterior) hooks measure about 90μ in length.

Proboscis-sheath. The proboscis-sheath is inserted at the base of the proboscis. There is no neck. In the male the sheath measured 1.4 mm. by 0.76 mm., and in the females 1.78 mm. by 0.7 mm. The central nervous system lies about the middle of the sheath.

Lemnisci. These are slightly more than twice the length of the proboscis-sheath.

Testes. The testes are situated just posterior to the proboscis-sheath, lie obliquely one in front of the other, and measure about 1.5 mm. by 0.66 mm.

Prostatic glands. There are, apparently, six very long cylindrical prostatic glands terminating immediately behind the posterior testis.

Eggs. These measure 78μ by 41μ ; they have no polar capsules.

Echinorhynchus bulbocaudatus, sp.n.

Very numerous specimens from a bush pheasant (*Centropus phasianus*). Townsville, Queensland, Northern Australia.

The females measured about 58 mm. in length, and in greatest breadth about 1.1 mm. The male (we had only one adult at our disposal) measured about 26 mm. in length, and the greatest breadth was 0.9 mm. The cuticle is smooth. The worms are long and cylindrical. In the female the terminal 3 mm. is oval and dilated, and the body ends in a point.

Proboscis. The shape of the proboscis varies from oval to sub-spherical. It is small and arises somewhat obliquely. The proboscis is separated from the body by a short neck (about 0.2 mm. long), which is devoid of hooks. When the proboscis is partly retracted, as it is in most of our specimens, the anterior portion of the body, overhangs its base and the cuticle is folded so as to resemble a frill or ruff. The proboscis measures about 0.5 mm. to 0.7 mm. in length, and 0.4 mm. to 0.5 mm. in greatest breadth. It is armed with numerous hooks, arranged radially and distributed in about twenty-eight antero-posterior rows, each composed of about nine hooks. The hooks in the fourth and fifth rows are the largest and measure about 45μ in length. Each hook is provided with a conspicuous, long, rectangular root, slightly hollowed out at its posterior margin.

Proboscis-sheath. This arises a little anterior to the base of the proboscis-like structure, that is, there is a short neck which is unarmed. In the male the proboscis-sheath measured 1.46 mm.

by 0.24 mm., and in the female 1.5 mm. to 1.6 mm. by 0.27 mm. The central nervous system lies in the anterior half of the sheath.

Lemnisci. These are rather more than twice the length of the proboscis-sheath and are massive; in the male they overlap the anterior testis.

Testes. These are situated obliquely one behind the other and they overlap; they lie about 0.7 mm. behind the proboscis-sheath. Each testis measures about 1 mm. by 0.6 mm. In an immature specimen, however, the testes were well separated from each other, and were situated more posteriorly.

Prostatic glands. These are, apparently, six in number, long and tubular, extending to the posterior testis.

Eggs. These measure about 60μ by 30μ and are without polar capsules.

Echinorhynchus clavula Dujardin, 1845, *nec* Hamann.

Three females and one male from the body cavity of a sea bream (*Sparus berda*). Townsville, Queensland, Northern Australia, 8.II.1920 (Dr. P. A. Maplestone). Also two males and one female from the intestine of a 'yellow tail' (*Trachurus declivis*), Australia, 8.II.1920 (Dr. P. A. Maplestone).

Echinorhynchus gadi Zoega, 1776.

SYNONYM:—*Echinorhynchus acus* Rud., 1802 (according to Lühe, 1911).

Four females and one male from the intestine of a haddock. Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone). Also one gravid female from the intestine of a pollack (*Gadus pollachius*). Port Erin, Isle of Man (Dr. Annett). Also very numerous males and females from a codling, North Sea, October, 1922 (Professor James Johnstone).

Lühe gives the size of the eggs as 76μ by 13μ , but in our specimens they were larger and measured 107μ by 24μ (average of 10).

Echinorhynchus truttae Schrank, 1788.

SYNONYM:—*Echinorhynchus fusaeformis* Zeder, 1803.

Six females and six males from a trout, 11.I.1923 (A. W. Noel Pillers, F.R.C.V.S.). The females varied in length from about 11 mm. to 19 mm. They are broadest near the anterior extremity,

the maximum breadth being 1.2 mm. The largest male measured 10 mm. in length, and had a maximum breadth of 0.86 mm.

Lühe gives the size of the egg as 100μ to 110μ in length by 23μ to 24μ in breadth; v. Linstow states that they measure 136μ to 140μ by 23μ to 26μ . The average of ten eggs in the body cavity of one of our females was 137μ by 26μ .

Three females and four males from the body cavity of a sea bream (*Sparus berda*), 20.9.1920.

Three females and one male from the intestine of a fish ('grunter'). Townsville, Queensland, Northern Australia, 3.10.1920 (Dr. P. A. Maplestone). In these specimens the lemnisci extended slightly beyond the extremity of the proboscis-sheath.

Genus. *Lueheia* Trav., 1919 (?).

Travassos recently (1923) described under the name *Lueheia lueheia* a species of *Acanthocephala* obtained from *Thamnophilus severus* and *T. guttatus* with the following characters:—'Body broad, thick, fusiform, having large folds, milky-white in colour, measuring about 7 mm. in the case of the male and 12 mm. in the female in length, by 1.2 to 1.8 mm. in greatest breadth; proboscis slightly globose, not invaginable in the adult, but retractile into the extremity of the body, measuring about 0.43 to 0.52 mm. in length by 0.38 to 0.46 in greatest breadth, furnished with 22 to 24 longitudinal rows of eight or nine hooks each; the hooks increase in size from the head to the more enlarged part; from thence, as far as the base, they grow progressively smaller; these hooks are comparatively strong, and of three chief types, the anterior hooks are delicate, those in the middle are very strong and U-shaped, and finally, those at the base are falcated.

Measurement of hooks:—

| Specimen. | Base. | Lamina. |
|-----------|-----------|-----------|
| 1 | 0.037 mm. | 0.034 mm. |
| 2 | 0.037 mm. | 0.042 mm. |
| 3 | 0.054 mm. | 0.045 mm. |
| 4 | 0.068 mm. | 0.059 mm. |
| 5 | 0.071 mm. | 0.059 mm. |
| 6 | 0.048 mm. | 0.054 mm. |
| 7 | — | 0.048 mm. |
| 8 | — | 0.048 mm. |

Neck absent; sheath of proboscis club-shaped, measuring about 0.78 to 1 mm. in length by 0.26 to 0.27 mm. in greatest breadth; lemnisci six in number, cylindrical, straight in the female, curved in the male, measuring more or less 1.9 to 2.8 mm. in length; testes ellipsoid, situated some distance from the sheath but in contact with, or partly over-lapping, the lemnisci, measuring about 0.7 by 0.3 mm.; prostatic glands in contact with the nearest testis, elongated, voluminous, measuring about 1.3 mm. in length; deferent canals showing symmetrical extensions to the level of the nearest third of the prostatic glands, and joining up at the level of the most remote third, to form a voluminous seminal vesicle, in the shape of a very thick Y which straightens out to form the ejaculatory canal. The ejaculatory canal and the ducts of the prostatic glands measure about 0.7 mm. in length; the copulative pouch is comparatively small; eggs bearing bacilliform nuclei and without polar capsules, measuring, in the median plane, 0.078 to 0.075 mm. in length by 0.028 to 0.31 mm. in greatest breadth; small egg-ejector 1 to 1.5 mm. long.

Habitat. Small intestine of *Thamnophilus severus* and *Th. guttatus*.

Specimens in the Oswaldo Cruz Institute, No. 1888, Angra dos Reis, Rio.

This species is very closely related to *L. inscripta* W., from which it is distinguished by a proboscis with a greater number of longitudinal rows of hooks, and a greater number of hooks in each row, the hooks themselves being also larger.

'The walls of the body appear less rugose, and in this species there is a difference in the structure of the peripheral stratum near the middle of the walls of the body, where it is clear in comparison with Westrumb's species, a difference appreciable even in specimens prepared whole. The differences in the male genital organs, which are not placed within strictly defined limits, can scarcely be observed in the very young male specimen of *L. inscripta*. It is interesting to note that while the two species exist side-by-side in the neighbourhood of Angra dos Reis, yet *inscripta* is rare and generally found as isolated specimens; the other is common and found in large numbers in every carrier.'

We have, unfortunately, been unable to obtain Travassos's

description of the genus *Lueheia* and no figures of *Lueheia lueheia* are given. Travassos apparently places the genus in the sub-family CENTRORHYNCHIDAE.

The somewhat reduced proboscis, which in the adult is not retractile within its sheath, are characters which ally the species to the OLIGACANTHORHYNCHIDAE, and especially to the genus *Oligacanthorhynchus* or the genus *Prosthenorchis*, but on the other hand the body is small, the proboscis bears numerous hooks and the worm is found in birds, characters which suggest affinities with the *Echinorhynchidea*.

The species *L. lueheia* is, however, unique in possessing six lemnisci instead of two. Until we know whether the proboscis sheath has a single or a double wall, where the sheath arises and how many prostatic glands are present, it is impossible to classify the genus satisfactorily, but in any case the presence of six lemnisci is a character sufficiently striking to identify the species; although there is, of course, the possibility (amounting, in this case, to probability) that the number '6' occurring in the description of the lemnisci is really a misprint for '2.'

ADDENDUM

Whilst this paper was in page proof Travassos' supplement to the Revision of the Family Gigantorhynchidae (1924) has come to hand.

In this paper he includes the genera *Micracanthorhynchus*, Travassos, 1916; *Empodius* Travassos, 1916 = *Heteroplus* Kostylev, 1914, n.p.; *Mediorhynchus* Van Cleave, 1916 = *Micracanthorhynchus* Travassos, 1917, in the family Gigantorhynchidae, and he defines the genera *Empodius* and *Mediorhynchus* as follows:—

Empodius. Proboscis armed with four transverse series of relatively large hooks and about fourteen longitudinal series of hooks with two hooks in each series. Neck sharply differentiated and armed with hooks having simple roots. Sheath of the proboscis not invaginable. Eggs with concentric membranes. Intestine of birds.

Mediorhynchus. Proboscis armed with from ten to twelve

transverse series of relatively small hooks and with about twenty longitudinal series of hooks, with five or six hooks in each series. Neck well differentiated and armed with small simple hooks. Hooks of the proboscis and neck situated in the centres of papilliform projections. Sheath of proboscis slightly developed. Proboscis not invaginable. Eggs with concentric membranes. Intestine of birds.

We agree that the two genera are distinguishable, but as we have found the proboscis invaginable in *E. segmentatus* we refer them to the Sub-order **Echinorhynchidea**.

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TETRACAMPOS WEDL 1861 AS A GENUS OF THE BOTHRIOCEPHALIDAE

BY

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In 1913, Southwell very briefly described from the Indian Siluroids *Ophiocephalus striatus*, *Labeo rohita* and *Wallago attu* a Cestode which, from the characters of the scolex, he identified as *Ophryocotyle bengalensis*, i.e., as one of the Davaineidae. In 1924, I described in some detail the anatomy of two species of Proteocephalids also from Indian Siluroids, viz., *Wallago attu* and *Macrones seenghala*, which I provisionally named *Gangesia wallago* and *G. macrones*, and I contended that the former species was almost certainly identical with Southwell's '*Ophryocotyle*' *bengalensis*. Southwell (1925) admits that my contention was correct, whence it follows that the specific name of my first species, assuming the retention of the genus *Gangesia*, should read *Gangesia bengalensis* (Southwell 1913).

This brief resumé of the history of this species serves to show that external characters, and especially scolex characters, cannot always be depended upon as a guide for the correct allocation of a new species in any modern system of classification. Southwell, however, has apparently not taken this view of the matter since in the communication referred to (1925) he revives an ancient undefined and inadequately-described genus first created by Wedl in 1861, viz., *Tetracampos*, and argues, once more chiefly on the basis of scolex characters, that *Gangesia bengalensis* is a second species of this genus, and that the name *Gangesia* must, therefore, lapse. This assertion that Wedl's species *Tetracampos ciliotheca* from the Nile Siluroid '*Heterobranchus*' *anguillaris* (= *Clarias lazera* according to Boulenger) was a Proteocephalid is very questionable. Wedl's other species (and new genus) *Marsypocephalus rectangulus* was

undoubtedly a Proteocephalid, as I have shown in a forthcoming paper (Woodland 1925), but there is every reason to believe, with La Rue* (1914), that *Tetracampas ciliotheca* was a Bothriocephalid, and I propose to give the reasons for that belief, but before doing so, it will be as well to state the evidence offered by Southwell in favour of *Tetracampas* belonging to the Proteocephalidae. This evidence, when examined, appears to consist solely of the general statement that 'the adult cestode parasites most common in fresh-water fishes belong to the genus *Proteocephalus*,' and the very superficial resemblance of Wedl's drawing of the scolex of *Tetracampas ciliotheca* to the scolex of *Gangesia bengalensis* (!). As regards the general statement, this is of course true enough, but Southwell omits to mention the fact that Bothriocephalids are also sometimes to be found in fresh-water fishes, and that at least one, and a very well-known one, viz., *Polyonchobothrium polypteri*, is to be found in a fresh-water fish from the Nile, viz., *Polypterus bichir*. I have also recently described (Woodland 1925) a new species of *Clestopbothrium*—*C. clarias*—from a Nile Siluroid, *Clarias anguillaris*. As regards Southwell's comparison of Wedl's drawing of the scolex of *Tetracampas ciliotheca* with the scolex of *Gangesia bengalensis*, I may point out that the hooks of the two scolices are very different in form, and that whereas those of *Tetracampas* are in four groups and vary in size, those of *Gangesia* form a single complete circle and are of the same size, and that Southwell's remark that 'it is impossible to decide from Wedl's figure and descriptions,' whether Wedl's four 'Lappen' ('Jeder Lappen besteht aus einem dünnwandigen, contractilen Parenchym und ragt an der Aussenseite des Kopfes als eine platte Scheibe hervor . . . Nach vorne sind diese Hautlappen (Bothridien van Beneden) näher an einander gerückt und umkreisen eine kuppelförmig hervorragende, bewaffnete Papille.') are 'really outgrowths from the head or whether they are true acetabula' is certainly no justification for his implied assumption that they *are* outgrowths which bear acetabula, such as exist in Proteocephalids. The foregoing constitutes the whole of the actual evidence offered by Southwell in support of his contention, though in further support of his view he has gone so far as to conclude that Wedl erred in

* *Tetracampas ciliotheca*, 'because of its ventral genital pore, ciliated embryo and two bothria, evidently belongs to the order Pseudophyllidea.' (La Rue.)

describing the genital openings as being situated on the ventral surface.

A careful examination of Wedl's figures and description affords, I think, decisive evidence that *Tetracampos ciliotheca* was a Bothrio-

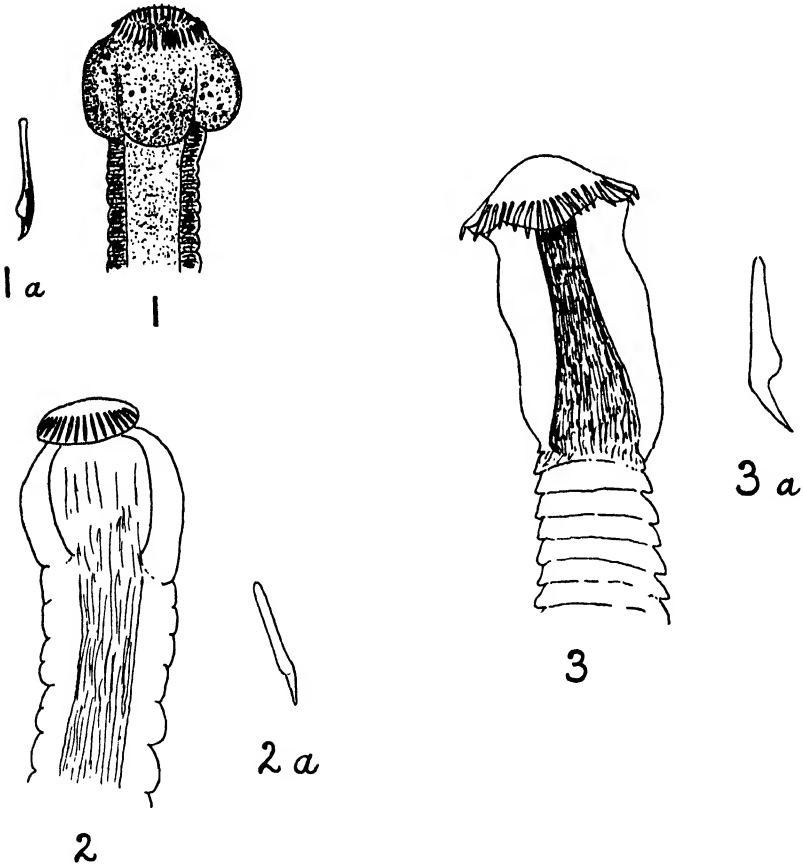


FIG. 1. Approximate copy of Wedl's figure of *Tetracampos ciliotheca*. Magnification about 100.

FIG. 1A. Approximate copy of Wedl's figure of a hook on the scolex of *T. ciliotheca*. Magnification unknown.

FIG. 2. Contracted scolex of *Clestobothrium clarias* Woodland. $\times 87.5$.

FIG. 2A. Hook from scolex of *C. clarias*. $\times 395$.

FIG. 3. Scolex of *Polyonchobothrium polypteri* Leydig. $\times 56$.

FIG. 3A. Hook of scolex of *P. polypteri*. $\times 180$.

cephalid. The hooks are very similar in form, number and arrangement to the hooks found on the crown of *Polyonchobothrium polypteri* (cf. figs. 1 and 3). In this latter species (fig. 3), as in *Tetracampos ciliotheca*, the hooks are arranged in four groups. In each group

in *T. ciliotheca* the number of hooks is usually nine (' of which the longest odd one is in the middle and the shortest pair on the outer side of each group '), while in *P. polypteri* the number varies between six and eight (Klaptocz 1906), and the hooks vary in size and in the position of the longer and shorter in each group, as in *Tetracampos*. In both species the general shape of the scolex is similar save that in *T. ciliotheca* the part below the crown of hooks is much shorter. This shortness is either natural and peculiar to the species or the drawing represents an unusually contracted specimen, similar to that which I have figured (fig. 2) for *Clestobothrium clarias*. This fig. 2 is a true representation* of a contracted scolex of *Clestobothrium clarias* (though the normal scolex is much more elongated—Woodland 1925†), and the general similarity between this representation and Wedl's figure of *T. ciliotheca* affords an explanation of all the general features shown in the latter. Wedl's species cannot be *Clestobothrium clarias* because in this latter the hooks are arranged in a complete circle, and are all of the same size, so markedly differing from the hooks of Wedl's species; neither can Wedl's species be identical with *Polyonchobothrium polypteri* because of the different sizes of the worms, among other reasons, but there is every reason to believe that Wedl's *T. ciliotheca* is a Bothriocephalid of about the same size as *Clestobothrium clarias* (my largest specimen of which measures 14.5 mm.; *T. ciliotheca* measured 10-15 mm. in length), but with the hooks similar to those of *P. polypteri* and possibly a shorter scolex. I have already quoted Wedl's description of the four scolex 'Lappen,' which are evidently the four walls bordering the bothrial or sucking grooves. Other typically Bothriocephalid features of *T. ciliotheca* are the shape of the anterior proglottids, the ventral position of the genital apertures (so conspicuous in these forms, even with an imperfect technique) and the ciliated embryophores enclosing the hexacanth embryos.

I conclude, therefore, that Southwell is mistaken in supposing that Wedl's genus *Tetracampos* has any connection with *Gangesia bengalensis* and *G. macrones*.

As regards Southwell's remarks on the systematic position of the Proteocephalidae this, of course, is a disputed subject, but I may

* As Dr. C. M. Wenyon can testify.

† This paper will provide my reason for including this species in the genus *Clestobothrium*.

say that for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the Tetraphyllidae, and that, with me, scolex characters count for very little, though even in this connection, Southwell appears to ignore the lobes upon which the suckers in this family are usually borne (*vide* Beddard 1913, pp. 8, 11, 12 e.g.).

I wish to acknowledge my indebtedness to Dr. H. A. Baylis for the kind gift of a number of specimens of *Polyonchobothrium polypteri*, and to Miss I. M. Bellis for assistance in connection with the literature.

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THE MEASURE OF HOOKWORM INFECTION IN COMMUNITIES

BY

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INTRODUCTION

In recent years the necessity for some measure of the degree, as well as the extent of hookworm infection in localities and communities, has been realised by a number of investigators, e.g., Darling (1922) and Cort (1924). It is an obvious but, nevertheless, largely ignored fact, that the percentage of individuals in whose stools eggs can be found is far from giving a true index to the severity of the hookworm situation, and yet, until very recently, this percentage has been accepted as the standard of measurement. The fallacy of this standard is, perhaps, nowhere more evident than in Bengal where, in spite of the fact that 70 per cent. or more of the population are infected, the individual infections are, on the whole, so light as to make hookworm disease in this province a comparatively unimportant problem. A correct estimate of the need for hookworm work and the judicious allocation of funds available for hookworm campaigns, as well as the strategy of campaigns, should depend on the degree as well as the prevalence of the disease. The relative value of different control measures and of different methods of treatment, also, can be correctly judged only by a consideration of the reduction in amount as well as in the incidence of infection. Hill (1923), for instance, showed that in certain areas in Porto Rico an intensive campaign reduced the percentage of infection from 87.2 to 34.1, but it reduced the egg output, which was used as a measure of the amount of infection, by 92.4 per cent.

METHODS OF MEASURING INTENSITY OF INFECTION

They may be classed as follows: (1) effects on host, (2) worm counts after anthelmintic treatment, and (3) estimation of the egg output in the faeces.

Clinical symptoms, haemoglobin percentage and eosinophilia are the principal factors used in measuring effects on the host. All workers agree that the estimation of the amount of hookworm disease on the basis of clinical symptoms is difficult and complicated by differences in individual resistance, age, conditions of life, and concurrent disease; by the personal element in the classification of symptoms and severity of cases; and by the difficulty in making anything more than a very rough classification into light, moderate and severe cases. Such a clinical classification is of value in giving supplementary data as to the effects of the disease under local conditions, and in demonstrating individual and racial resistance, but it is of very little value, *per se*, as an indication of the degree of hookworm infection in a community. One might as well attempt to determine elevation on a mountain by reference to the permanent snow line, without consideration of other circumstances.

The haemoglobin content of the blood, as a measure of the degree of hookworm infection, is of little or no value in individual cases, although some authors, e.g., Darling, Barber and Hacker (1920), maintain that when sufficiently large numbers are averaged the amount of anaemia is proportional to the number of worms. Darling (1922) and Sawyer and Sweet (1922) have suggested definite ratios between the number of worms harboured and the percentage loss of haemoglobin. The haemoglobin content, however, is affected by so many factors such as sex, work, age, malnutrition, and such blood diseases as malaria, kala-azar, etc., that it can be used as a measure of hookworm infection only within wide limits. The process of elimination of other causes of anaemia is long and tedious, and in light cases there is usually no measurable drop in haemoglobin content. In a study of 100 individuals in the Alipore Central Jail, Calcutta, 67 of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gram of faeces, no difference in haemoglobin percentage between the infected and non-infected individuals could be found. The average for the uninfected ones,

according to the Tallquist scale, was 82.3, and for the infected ones 83. Two uninfected and only one infected case fell to 60 per cent., whereas one infected and one uninfected case reached 95 per cent. Darling (1922) shows that, in especially selected homogeneous groups, it requires fewer worms to cause a given loss of haemoglobin in a woman than in a man, and still fewer in a child, and also that a given number of *Ancylostoma duodenale* produces more anaemia than a similar number of *Necator americanus*, a fact which is now quite generally recognised. It is very probable that, as the number of worms increases, the haemoglobin content decreases at an accelerating rate, since it would become increasingly difficult for the patient to make good the loss produced by the worms.

Eosinophilia, as an indication of hookworm disease, is open to much the same criticisms as is the estimation of haemoglobin, since hookworm is only one of many causes of this condition. Practically all helminth infections produce more or less eosinophilia. Moreover, McVail (1922) observes that the eosinophilia in ankylostomiasis is not proportional to the number of worms present, even in uncomplicated cases, and he shows that kala-azar, and to a less extent malaria, is a powerful factor in reducing the eosinophilia due to helminthiasis.

The counting of worms passed after anthelmintic treatment, as a method of estimating the degree of infection in a community, is of unquestionable value when it can be properly carried out, but the difficulties involved are in most instances practically insurmountable. Only a small number of persons, and these especially selected ones, who can be relied upon to save all stools, can be examined in this way at a reasonable cost. The method requires a trained personnel, and cannot be left to subordinates; carelessness on the part of patients or laboratory staff, the partial failure of anthelmintics, and the loss of worms by maceration are all factors which interfere with the accuracy of the results. Even with every precaution which we have found practicable in the case of our hospital patients, we have not infrequently found lightly-infected cases to become microscopically cured after treatment, without finding any worms in the stools. It has usually been assumed that the washing of stools for 48 to 72 hours after treatment is sufficient to recover all of the worms passed, but even when a saline purge is given we have found

worms in stools as late as the sixth day after treatment, and quite frequently on the fourth day. Occasionally the stools have been negative on the second or third day, and again contained worms on the third or fourth day. For these reasons it is obvious that, however desirable a method for estimating degree of infection the worm count may be from a theoretical standpoint, it is certainly not in most instances practicable, and is always expensive.

The estimation of degree of infection by the egg output in the stools has very distinct advantages in the way of simplicity and practicability, providing that the egg output actually indicates the amount of infection. Even if this should prove to be true only to a limited extent and only when considerable numbers of individuals are averaged together, the knowledge of the number of eggs being deposited on soil, as Payne, Cort and Riley (1923) and Hill (1923) have pointed out, is, in itself, an important bit of information from the point of view of the spread of the disease. It is the egg output, and not the number of worms harboured or the clinical symptoms, which measures the public health menace.

ESTIMATION OF EGG OUTPUT

A number of different methods of estimating the actual or relative numbers of eggs in faeces have been utilised by different workers. Most of the methods of microscopic diagnosis can be used to give rough quantitative as well as qualitative information, but few of them are well adapted to give accurate information on this point. One of the first exact methods was Lane's (1918) 'standardising count,' which is Howard's centrifugal concentration technique reduced to accuracy of measurement, but this was not used for determining intensity of infection in groups or communities of people. The method devised by Stoll (1923a) is the only one which has been utilised in this way on a large scale. It is very simple, consisting merely of accurate dilution of a weighed sample of faeces in a decinormal NaOH solution, to clarify the fatty constituents of the faeces, and the accurate counting of a carefully-measured sample of the dilution. Lane (1924) has suggested the use of his direct centrifugal flotation method for this purpose, but, excellent as it may be for diagnosis, if the necessary apparatus is available,

it does not seem to me to be well adapted for quantitative work since, except where less than 500 eggs per gram are involved, the difficulty and tediousness of counting the great number of eggs thrown on the slide would counterbalance the advantage in reduced area of examination. It would be necessary, if numerous eggs were found, to repeat the process, using a much smaller quantity of stool, which would involve both time and inaccuracy due to the difficulty of measuring, say, 0.1 c.c. of stool.

The favourable results obtained by the use of Stoll's egg-counting method in Porto Rico led to a trial of it, with a few modifications, in Bengal. We have modified Stoll's method (1) by diluting the 3-gram sample of faeces to 90 c.c. instead of 45 c.c.; (2) by counting the eggs in a 0.3 c.c. sample of the dilution instead of 0.15 c.c.; and (3) by examining the preparation uncovered. There are several advantages in these modifications. In searching for eggs on an area of about two square inches on an uncovered slide, marked off by means of a glass pencil, it was found that 0.3 c.c. of the faecal suspension was necessary, under tropical conditions, to prevent the preparation from partial drying before the examination was complete. In order to get a sufficiently clear field for examination of ordinary stools with this quantity of the suspension, a dilution of 1 to 30 instead of 1 to 15 was necessary. The greatest advantage of using the larger amount of fluid and examining the preparation uncovered lay in the ability lightly to blow aside the flocculent masses of debris which often tend to hide the eggs, by gently puffing on the slide while the examination is actually in progress. Camouflage of eggs by debris is the most important source of error in all the techniques in which accurate measurements of material are made. Lane (1923, 1924) gives convincing evidence of the loss of eggs by camouflage. Maplestone (1924), in testing Stoll's method, nearly always obtained higher counts per gram when the faeces were further diluted before the egg count was made, obviously due to overlooking of eggs as the result of concealment in the more concentrated samples. By using a suspension in decinormal NaOH on an open slide with 0.3 c.c. of fluid spread over an area of 2 square inches, it is ordinarily possible, by gently blowing on the slide while making the examination, to see practically 100 per cent. of the surface of the slide with sufficient clearness to render the eggs easily visible. The eggs are heavier than

the flocculent material which makes up the great bulk of the débris on the slide, and, therefore, rest on the slide and remain visible as the overlying material is puffed aside. Furthermore, one can almost instantaneously determine whether or not an object which resembles an egg is such, since its position can be slightly changed or it can be rolled over by the same gentle puffing process. In very concentrated formed stools, we sometimes find it necessary to divide the 0.3 c.c. sample on two slides and dilute them further.

There are a few possible sources of error in this method which may be briefly commented on. In the first place, the selection of a 3-gram sample of faeces should, when possible, be made from an entire stirred stool, since the number of eggs contained in different parts is not always the same. Making duplicate counts on two samples from different parts of a single stool, the widest differences we obtained were counts of 700 and 900 eggs per gram on one sample and 1,600 and 1,800 on the other. After stirring this stool, examination of a third sample gave two counts of 1,200. In field work it is usually not practicable to get entire stools, and counts must be made on the samples submitted. As will be subsequently shown, however, the error arising from this, in individual cases, is neutralised when 50 or 100 samples are averaged.

We have tested a number of diluting fluids but found that the decinormal NaOH solution gave clear fields and more readily visible eggs than any other fluid. Addition to the NaOH of 1.5 per cent. NaCl had the effect of causing the faecal débris to clump together into large light flocculent masses which could be blown about, leaving a beautifully clear background on which the eggs showed up with striking clearness, but the occasional entanglement of eggs in these masses reduced its accuracy.

The thorough mixing of the samples in homogeneous suspensions is sometimes slow and difficult, and it is easy to overlook small masses of faeces which have failed to disintegrate. Unless carefully watched, this is one of the most fruitful sources of error. When available, a mechanical shaker is of great advantage. Settling of eggs in the diluting fluid must also be carefully guarded against; the stopper of the flask should be removed and the sample withdrawn immediately after a thorough and vigorous shaking. Even a few seconds' delay entails inaccuracy. We have found that the samples

can be withdrawn more quickly and accurately into rubber-bulb pipettes of drawn glass tubing marked at the 0.3 c.c. level, than into bacteriological pipettes. Only the required amount of fluid is sucked into the pipette, and all of it expelled on to the slide.

The NaOH solution does not appreciably change the appearance of the eggs of hookworms, *Trichuris*, *Hymenolepis nana*, or *H. diminuta*, but *Ascaris* eggs have the rough albuminous coat more or less completely dissolved off and thus often look quite different from the normal eggs, especially in the case of unfertilised ones. *Taenia* eggs undergo a peculiar change in that the embryophore swells to a diameter of from 50 to 60 μ , leaving a much-enlarged clear space between it and the embryo; the latter shrinks somewhat and assumes a characteristic elongated form.

Recounts of the same slide, duplicate counts from the same suspension, and counts on higher dilutions have shown that camouflage of eggs is practically done away with by the method here described. Lane warns against the loss of eggs held on the surface of a film too deep to be in one optical plane and in which only the bottom is searched. Apparently this rarely happens in a decinormal NaOH solution since I have several times gone over the surface of a slide containing several hundred eggs without finding a single egg. The entanglement of eggs in flocculent masses occasionally occurs, though much more frequently with *Ascaris* than with hookworm eggs. It usually takes a little time for the débris on the slide to clump, a process which takes place much more extensively in some stools than in others; consequently, it only rarely happens that the eggs do not have time to settle. The blowing process also aids in liberating them. There is no doubt but that some loss of eggs does occur in these ways, but even if there were a constant loss of, say, 10 per cent. of eggs, it would be of little consequence, since what is desired is not so much an absolute knowledge of the number of eggs as a comparative measurement of the egg output.

That the method here described gives a good comparative measurement of eggs per gram of faeces is shown by the uniformity of counts which are obtained from examinations of two different samples prepared from the same stool. Where the average count on the slide is 10 or less, in about 80 per cent. of several hundred duplicate examinations, the counts were identical or within one

of each other, and, therefore, as close as possible to the average. In another 16 per cent. the counts were two numbers apart, whereas in only about 4 per cent. were the counts three numbers apart. Where the average slide count is between 10 and 100, in 35 per cent. the two counts came as near as possible to the average, in another 44 per cent. they were not over 10 per cent. from the average, whereas in only 8 per cent. were they more than 15 per cent. from the average. Where the average count exceeded 100, 87 per cent. of the duplicate counts fell within 10 per cent. of the average and none over 15 per cent. from it.

In nearly every instance in which there was any considerable discrepancy in the two counts a clumping of the eggs was observed, evidently due to their being held together by strands of mucus which had not been broken up in the shaking, in spite of an apparently homogeneous suspension. This clumping was also observed by Davis (1924), but with our technique we have only rarely obtained as irregular duplicate counts as Davis records in many of his cases; undoubtedly he was dealing with mucous stools.

In a series of about 600 faecal samples received from the Alipore Central Jail, counts have been made on two different slides prepared from a single suspension made from samples collected in quarter-ounce faeces-tins. The results which have been obtained from these counts compare very closely with those obtained from examinations of two separately prepared suspensions. This indicates that the differences in the counts are due not to variations in different parts of a stirred stool, but to errors in the counting technique. It appears, therefore, that a single suspension made from a stirred stool gives a sufficiently fair sample of the entire stool.

RELIABILITY OF EGG COUNTS AS AN INDICATION OF DEGREE OF INFECTION

It is now important to know the amount of variation which occurs in the eggs per gram of faeces in individuals according to the consistency of the stool, and from day to day. To get some light on this we studied the egg content of the stools of 36 hospital patients on from 3 to 22 different days, and made duplicate egg counts on separately prepared suspensions from 194 stools. By classifying the

stools as liquid, mushy, semi-formed and formed, and comparing the egg counts of these several groups in the case of each individual, it soon became apparent that, roughly speaking, the formed stools contained twice as many eggs and the liquid stools half as many or less, as the mushy stools. This compares fairly closely with Stoll's findings in Porto Rico (1923b). It was evident, therefore, that if intensity of infection were to be measured by egg counts, the factor of consistency would have to be considered.

Since, in India, mushy stools are normal and formed ones are rare, we accept the count on mushy ones as normal and correct the counts on formed and liquid stools by dividing or multiplying by 2. Such counts we refer to as 'corrected counts.' In a paper which has recently come to hand, Stoll (1924) arrives at exactly similar conclusions, except that he accepts formed stools as normal and multiplies the counts on mushy and liquid stools by 2 and 4 respectively, to bring them to 'basis of formed stool.'

Our counts on these preliminary 36 patients showed, however, that even when the consistency of the stool does not vary, there is a surprising variation in the egg output per gram of faeces on different days. In case 22, for instance, considering only the mushy stools, there was a maximum variation from 250 to 1,100 eggs per gram, in case 29 from 500 to 1,250, in case 32 from 250 to 1,000, and in case 35 from 50 to 350. These are the most extreme cases; in most instances, if the consistency of the stool is taken into consideration, the variation is much less. There appears to be a much more marked tendency to vary in some individuals than in others. Stoll (1924), in a study of the egg output of two individuals, for 15 and 40 days respectively, found a similar day-to-day variation. In one of his cases the mushy stools varied from 1,000 to 2,600 and in another from 430 to 800, whereas the formed stools in the latter case varied from 400 to 1,330.

An attempt was made to get 24-hour samples of stools and to calculate the total daily egg output for 24 hours by means of the egg count and stool weight, since it seemed probable that the amount of the stool would to some extent counterbalance the variations in eggs per gram. Our results, however, failed to show any such counterbalancing tendency, since it just as frequently happened that a low egg count was accompanied by a small 24-hour output of stool

as the reverse, thus giving a greater variation in the total egg output than had been found in the number of eggs per gram. Stoll's (1924) tables show a similar lack of correlation. The most obvious reason for this appears to be that the extent to which the bowels are emptied on each day varies, even if the habits are fairly regular. In most of my cases the hour at which the stools are passed each day varies considerably, so it occurred to me that better results might be obtained by weighing only a single stool each day and keeping a record of the time between the last previous stool and the one examined. In this way we should know the number of hours during which the faeces and the eggs contained in them had been accumulating and could calculate from this the number of eggs produced in 24 hours. 112 stools from 23 different cases were examined in this way, but practically the same amount of variation was found in daily output as when 24-hour outputs of faeces were weighed without reference to time of stools, undoubtedly due to the same factor of completeness of evacuation of the bowels.

As Stoll has pointed out, it is only when the total daily output of eggs, calculated from eggs per gram and weight of stool, is averaged for at least three days that the coefficient of variation is reduced to a low level. For one-day examinations the egg count by itself gives less variable results than the calculated total egg output. Since, under field conditions, the collection and weighing of stools for three days on any considerable number of individuals is out of the question, for the same reasons that worm counts are impracticable, reliance must be placed on the egg counts alone, even though some inaccuracy is involved. Stoll has shown that in the two cases he examined, which were of widely different types, the total egg outputs showed a relation of 5.4 : 1, whereas the average corrected egg counts per gram showed a relation of 3.3 : 1. The failure of the egg counts to show a correct relationship is, of course, due to differences in food habits and consequent daily amount of faeces in which the eggs are distributed. We believe, however, that in more or less homogeneous groups, such as tea garden coolies, mine labourers, etc., habits are sufficiently alike for the corrected egg count, if averaged for three days, to give a reasonably good index of the relative egg output of different individuals. Since the egg counts of individuals approach a level when averaged for three or four days, it is obvious that in

determining the degree of infection of a group or community by counts on 50 or 100 individuals, single egg counts are quite sufficient, since variations would automatically be blotted out in the consideration of such numbers.

To test this point a study was made of 100 prisoners in the Alipore Central Jail, with the kind co-operation of the Superintendent, Lt. H. A. Young, I.M.D. A double count was made from a single suspension on two separate occasions, about a week apart. Most of the infections found were extremely light, so that although 67 were shown to be positive for hookworm, by the Kofoid and Barber technique, only 45 positives were found by examination of two slides prepared for egg counts on the first examination, and 44 on the second. Eleven which were negative on the first examination were positive on the second, and 12 which were positive on the first were negative on the second. Of these 23 cases, 12 showed only a single egg on four slides, six more gave an average of one egg per slide on the positive examination, and the remaining five gave average counts of from 1.5 to 2.5 on the positive examination. In spite of the high percentage of these low counts, which would tend to increase the probable error in the two counts, the average number of eggs per gram of faeces on the first examination was 282 and on the second 257, a deviation of only 4.6 per cent. above and below the average of the two. This compares quite favourably with the deviations of 3.9 per cent. and 3.3 per cent. from the average, which were found in the first and second slides in the first and second examinations respectively. This justifies the conclusion that a single egg count on a fair number of individuals gives a reasonably good estimate of the average egg output of that group.

Owing to the fact that we have not found it practicable to control our hospital patients sufficiently so that the preservation of all stools passed after anthelmintic treatment could be depended upon, we can give no reliable statistics on the relationship between egg counts and worms harboured. In cases in which we have reason to believe that all the stools were saved, the number of eggs per gram per female worm usually falls between 8 and 20. In one instance, however, in which duplicate counts were made for three successive days, without finding any eggs at all, although the case was positive by flotation, four female *Necators* were passed. In another case which

passed 16 female *Necators* two eggs were found on each of two slides on one day and no eggs on duplicate examinations on two subsequent days; in this case something must have inhibited oviposition on these two days. There is likely to be less variation of this kind in the field than in a hospital, where alterations in diet, drug treatments, and concurrent disease may influence both the quantity of the stool and the oviposition of the worms.

That the correlation between egg count and worms harboured is not close in individual cases is evident from the day to day variations in the count. Mhaskar (1923) gives a table of 30 cases which purports to show that there is no correlation at all. Darling (1922) on the other hand, gives a table in which a distinct correlation is shown. Smillie (1921) and Stoll (1923b) also find a correlation. On purely theoretical grounds one is forced to the conclusion that, other things being equal, there *must* be some relationship between egg output and number of worms harboured. For instance, if a patient harbouring 10 female worms produced, on successive days, 100, 500, and 200 eggs per gram of faeces, is there any reason to doubt that if he harboured 20 female worms, other conditions being the same, he would pass on each of these days approximately twice as many hookworm eggs? It is reasonable to assume, then, that when the egg output of a large number of representative individuals is averaged together, this number gives a sufficiently accurate estimate of the degree of infection so that it can be used for comparison of different groups of individuals living under similar conditions and having similar food habits, or of the same groups before and after treatment, or for the establishment of control measures. The average eggs per gram is a less accurate guide in comparing groups living under quite different conditions and having widely different food habits, but even here, within wider limits, rough comparisons can be made. This is, however, of far less value and importance, for practical purposes, than the comparison of different groups of a single area by age, sex, occupation, etc., and the comparison of such groups at different times for the valuation of the effectiveness of control measures.

ESTIMATION OF INFECTION INDEX

Although Cort (1924) suggests the substitution, in surveys, of the egg counting method for the routine faecal examinations now generally used, and describes hypothetical cases which show its advantage, it seems to me that there is fallacy in accepting either the degree of infection as determined by worm or egg counts, or the mere percentage of incidence of infection, as an index of the amount of hookworm infection in a community, or of the benefits derived from treatment or control measures. For example, let us suppose that in two communities both living under climatic and soil conditions favourable for the propagation of hookworm, the number of eggs per gram of faeces averages exactly the same, but that the sanitary conditions and habits of the people differ. In one community the majority of the people are sanitary in habits and the hookworm infection is largely confined to a few families who are backward and careless in habits, while in the other community sanitary conditions throughout are not so good and the infection is more uniformly scattered through a high percentage of the people. In such a case it is clear that the two groups should not be placed on a par, as would be the case if only the degree of infection for the group, based on egg output, were considered; nor should the condition of the first community be considered as far superior to that of the second as the difference in percentage of infection would probably place it. From the standpoint of the general effect on the community, the probable spread of the disease, and the sanitary conditions indicated, it is important to take into consideration the number of individuals among whom the egg output is divided. Certainly the higher the percentage of individuals who are scattering a given number of hookworm eggs daily, the greater the opportunity for the spread of the disease, and the more important it is that control measures should be inaugurated. One hundred individuals each with an output of 100 eggs per gram of faeces certainly constitute a greater menace to the community than ten individuals each with an output of 1,000 eggs per gram, or one individual with an output of 10,000 per gram, since, although the total number of eggs produced is the same in each instance, the extent to which they are scattered is largely proportional to the number of persons who are passing them, and the more they are

scattered the more opportunity there is likely to be for the larvae which develop from them to gain access to new hosts. The incidence of infection, then, rather than the degree of infection, is the correct measure of the extent to which the entire community has been, and is likely to be, exposed to the infection, whereas the degree of infection rather than the incidence of it is a rough measure of the extent to which individuals have been, and are likely to be, exposed, and of the facility with which infection can occur, under the climatic and soil conditions of the locality, when carelessness in habits permits it.

It seems to me, therefore, that both factors must be taken into consideration in order to arrive at a true hookworm infection index. To do this I have tried various ways of combining the incidence and degree of infection, as indicated by eggs per gram of faeces, to obtain a number which would give a true relative index in various actual and hypothetical cases, as judged by a common-sense consideration of all the facts involved. Such an index number can, I think, be obtained by taking the square root of the product of the average eggs per gram, multiplied by the percentage of infection, or, alternatively, by taking the square root of the product of the egg counts, averaged for the infected individuals only, multiplied by the square of the percentage infected, i.e., by the equation :

$\sqrt{\frac{e.p.g.}{100} \times \%^2} = I$, where *e.p.g.* stands for average eggs per gram of the infected individuals ($\frac{e.p.g.}{100}$ being the average of the eggs counted on the slides), % the percentage infected, and *I* the resulting infection index. For example, if 50 of 100 individuals have an average of 400 eggs per gram by corrected counts, the other 50 having none, the equation would be : $\sqrt{\frac{400}{100} \times 50^2} = 100$, which is the infection index. The three hypothetical cases mentioned above of a 100 per cent. infection with 100 eggs per gram, a 10 per cent. infection with 1,000 eggs per gram, and a 1 per cent. infection with 10,000 eggs per gram, are all on a par on the basis of degree of infection for the group ; they stand in the ratio of 100 : 10 : 1 on the basis of incidence of infection ; while their infection indices work out at about 100 : 32 : 10, which seems to come much nearer their true relationships. It will be seen, however, that this method of calculation

gives correct results only if all the infections are uniform, since the average implies that the egg output is evenly divided among all the infected individuals, which is seldom the case. To get a correct estimate, therefore, the entire group of infected individuals should be broken arbitrarily into sub-groups according to the size of the egg counts, and the infection index for each sub-group separately figured and then all of them added together. For example, in a community with a 60 per cent. infection, 20 per cent. with egg counts of 100 to 500 (average 300), 20 per cent. with counts of 500 to 2,100 (average 1,000) and 20 per cent. with 2,100 to 5,100 (average 3,000), the infection index, if figured for the entire group, would be :

$\sqrt{\frac{1433}{100}} \times 60^2 = 228$, whereas if figured for each group separately, the infection index works out as follows: $\sqrt{\frac{300}{100}} \times 20^2 + \sqrt{\frac{1000}{100}} \times 20^2 + \sqrt{\frac{3000}{100}} \times 20^2 = 209$. We consider as very satisfactory the grouping used by Payne, Cort and Riley (1923), according to the following numbers of eggs per gram: 1-599, 600-2,099, 2,100-5,099, 5,100-11,099, and 11,100 up.

Table I gives the infection index, as worked out on a number of actual cases, based on my own work in Bengal and on statistics given by Payne, Cort and Riley (1923), and Hill (1923), in Porto Rico. It should be noted, however, that the Jute Mill statistics are not entirely correct, since the entire percentage of infection was not determined by a concentrative method, and therefore, as the egg counts run very low, a considerable number of light infections would probably be passed over by the egg counting technique, as was shown by the Alipore Jail investigation mentioned above. It is necessary, therefore, that the egg-counting method be supplemented by a concentrative technique in order to discover the light infections which would otherwise be missed. In calculating the infection index those cases which are positive by the concentrative method only, and negative on two egg-count slides, can be calculated arbitrarily as having 25 eggs per gram.

The method we have adopted, therefore, as a routine for determining the infection index, and which we recommend for general use, is as follows :—

TABLE I.

| | Number Examined | % with 1-599 e.p.g. | Average e.p.g. | % with 600-2099 e.p.g. | Average e.p.g. | % with 2100-5099 e.p.g. | Average e.p.g. | % with 5100-11099 e.p.g. | Average e.p.g. | % with 11100 or more e.p.g. | Average e.p.g. | Total % infected | Average e.p.g. for the entire group | Index of Infection |
|---|-----------------|---------------------|----------------|------------------------|----------------|-------------------------|----------------|--------------------------|----------------|-----------------------------|----------------|------------------|-------------------------------------|--------------------|
| Cookies in Jute Mills and Coolie Lines ... | 143 | 36† | 260 | 18 | 1020 | 4 | 3100 | 1 | 5700 | ... | ... | 58† | 420† | 140† |
| Cookies in Jute Mills and Coolie Lines ... | 48 | 19† | 275 | 17 | 1160 | 2 | 3400 | ... | ... | ... | ... | 38† | 310† | 100† |
| Cookies living outside Jute Mill ... | 98 | 44† | 242 | 14 | 830 | ... | ... | ... | ... | ... | ... | 58† | 220† | 109† |
| Prisoners in Allipore Jail (1st exam.) ... | 100 | 56** | 133 | 9 | 810 | 1 | 3200 | 1 | 8500 | ... | ... | 67 | 272 | 106 |
| Prisoners in Allipore Jail (7 days later) ... | 100 | 57** | 144 | 7 | 1040 | 2 | 3320 | 1 | 6450 | ... | ... | 67 | 286 | 110 |
| Cases in Porto Rico, Area C (Payne, Cort and Riley) ... | 92 | 17 | 300* | 16 | 1350* | 25 | 3600* | 20 | 8100* | 17 | 15000* | 96 | 7740 | 690 |
| Cases in Porto Rico Areas (before treatment) (Hill) ... | 282 | 28 | 300* | 27 | 1350* | 17 | 3600* | 9 | 8100* | 6 | 15000* | 88 | 2820 | 408 |
| Cases in Porto Rico Areas (after treatment) (Hill) ... | 282 | 25 | 300* | 6 | 1350* | 3 | 3600* | 1 | 8100* | ... | 15000* | 35 | 215 | 92 |

* These are means instead of averages, data for the latter not being available.

† These figures are too low, since no concentration method was used to discover infections too light for detection by the egg-counting method.

** These include 23 cases detected by concentration method, but negative by egg-counting method. These infections were arbitrarily figured as having 25 e.p.g. The figures in all cases are given to the nearest integer.

(1) Determination of the incidence of infection by a concentrative technique. In my experience the Kofoid and Barber method has given the most uniformly satisfactory results ; according to tests we have made it is more accurate than the Willis method or any of the usual centrifuge methods. If the necessary equipment is at hand the published evidence in favour of Lane's direct centrifugal flotation method indicates it as the method of choice, but since our centrifuges are not adapted to this method I have not had an opportunity of trying it myself. Neither the Kofoid and Barber, nor the Willis methods are reliable for light *Ascaris* infections ; to detect these we have found Lane's levitation method the most satisfactory.

(2) Determination of eggs per gram, by examination of all positives, by the modification of the Stoll egg-counting technique here described. By preference two slides should be examined and averaged, and the count corrected according to the consistency of the stool ; if, however, 50 or 100 specimens are examined, the total averages, though not the individual counts, will be very nearly correct if only one slide is examined of specimens showing two or more eggs. Specimens found positive by the concentrative technique, but negative on two egg-count slides, may be arbitrarily calculated as having 25 eggs per gram.

(3) Determination of the infection index by the equation :

$\sqrt{\frac{e.p.g.}{100}} \times \%^2 = \text{Infection Index}$, where *e.p.g.* stands for the average eggs per gram of the infected individuals, and where the equation is separately figured for different groups showing different degrees of infection, as suggested above.

In conclusion I take pleasure in acknowledging the painstaking and reliable help given by my assistant, Dr. A. K Mukerji.

SUMMARY

1. Recent investigations have shown the necessity for the measurement of the degree of hookworm infection as well as its incidence ; such a measurement is of value from the point of view of the urgency, nature and valuation of methods of treatment and control.

2. Degree of infection may be measured by clinical symptoms, haemoglobin content, eosinophilia, worm counts after treatment, or by estimation of egg output in the faeces. The first three are not reliable, and the worm counts are too difficult, impracticable and expensive for use on a large scale.

3. Estimation of egg output is simple and practical, and is of value in itself as an accurate measure of potential soil pollution whether or not it indicates accurately the number of worms harboured. A modification of Stoll's egg-counting method is described and recommended for general use. Uniformity of duplicate counts indicates that it gives a good comparative measurement of egg output.

4. The consistency of the stool must be taken into consideration in estimating the egg output from the number of eggs per gram of faeces. Counts on formed or liquid stools, where mushy stools are normal, as in India, can be corrected by dividing or multiplying by 2. Day-to-day variations in eggs per gram are considerable, but the counts approach a level when averaged for three or more days. Consideration of the quantity of the stool does not lessen the variation unless averaged for at least three days, and is, therefore, not practicable in field work. The corrected egg count alone must be relied upon, and in more or less homogeneous groups we believe that this gives a reasonably good indication of the relative degree of infection in different individuals. When averages of large groups are being considered, single egg counts of individuals are sufficient.

5. In individual cases the correlation between egg counts and number of worms harboured is not very close, but when the egg counts for a group are averaged, a fair estimate of the relative numbers of worms harboured can be obtained, especially when homogeneous groups, or the same groups at different times, are compared.

6. It is not advisable to measure hookworm infection in a community by the degree of infection alone ; the incidence should also be considered, since the higher the percentage of individuals who are scattering a given number of eggs, the greater the danger to the community. The incidence of infection is a measure of the extent to which the entire community is exposed to infection ; in a general way it measures sanitary conditions. The degree of

infection, on the other hand, is a measure of the facility with which infection can occur under the climatic and soil conditions of the region when carelessness in habits permits it.

7. A good infection index can be obtained only by taking both factors into consideration. This can be done by means of the equation : $\sqrt{\frac{e.p.g.}{100}} \times \% = \text{Infection Index}$, where *e.p.g.* stands for average eggs per gram of infected individuals only. This equation should be separately figured for different groups falling into certain arbitrary divisions according to number of eggs per gram, and all of them added together.

8. It is recommended that the infection index in survey work be determined as follows : (1) determination of incidence of infection by a concentrative technique ; (2) estimation of the degree of infection by means of egg-counts ; and (3) determination of the index of infection by the equation given above.

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A NEW VARIETY OF *ANOPHELES MARSHALLI* THEOBALD FROM THE BELGIAN CONGO

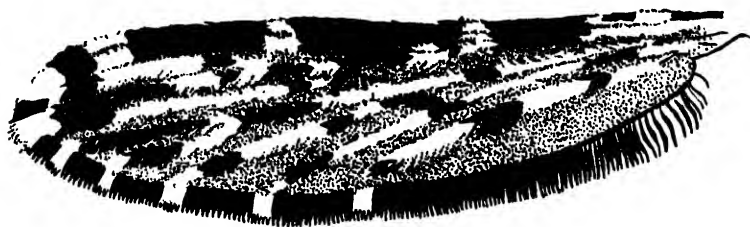
BY

A. M. EVANS, M.Sc.

(Received for publication 9 May, 1925)

Anopheles marshalli var. *moucheti* var. n.

FEMALE. *Head* with upright forked scales pure white anteriorly, black posteriorly; forwardly projecting tuft of long white scales reaching well beyond the base of the clypeus. *Palpi* with three white bands, the proximal narrow, the two distal bands very wide, equal in length, and separated by a black ring one-quarter to one-half of their length. *Antennae* with white scales on the second segment,



1 millimetre

A.M.E. 4

FIG. 1. *Anopheles marshalli* var. *moucheti* var. n. ♀ wing.

hairs of whorls white. *Thorax*: prothoracic lobes with blackish bristles, mesonotum with long, white, narrow, curved scales. *Abdomen* with dark integument and light brown hairs. *Wings* with white and dark scales disposed as shown in the illustration (Fig. 1). Typical plume scales from distal dark area of upper

fork of second vein (Fig. 2, A) with five striae and greatest width from one-fourth to one-fifth of the total length,* lateral squames from distal dark area of third vein (Fig. 2, B) mostly with five widely-separated striae, and greatest width one-fourth of the length. Legs black scaled; in all three pairs the tibiae and first three tarsal segments with narrow, but distinct, apical white rings. Hind legs with apical white ring also on fourth tarsal segment, mid legs with traces of pale scales apically on this segment. Length of white ring on hind metatarsus about equal to its greatest width, length of succeeding rings, progressively slightly shorter.

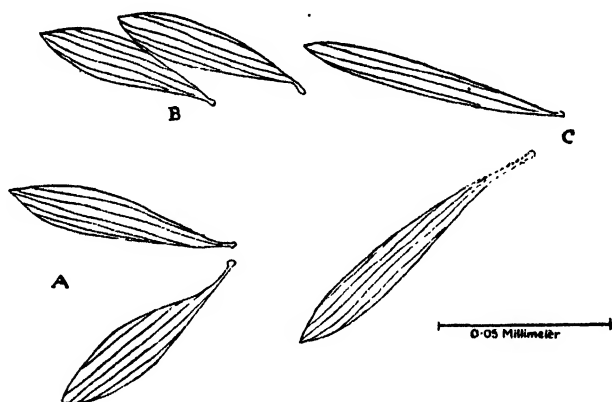


FIG. 2. *Anopheles marshalli* var. *moucheti* var. n. Wing scales. A—Plume scales from upper branch of second vein; B—Lateral squames from distal dark area of third vein; C—Plume scales from stem of second vein near fork.

Wing length: c. 3 mm.

MALE. Palpi with long segment black-scaled with narrow, apical, pale ring; last two segments white scaled with narrow basal black rings. Antennae with hairs of whorls whitish internally on proximal segments. Colouration as in the female.

The wing markings are subject to a certain amount of variation; the third pale area involving the costa and first vein may be as short on both veins as that shown on the costa in the illustration (Fig. 1), or on both veins as long as, or slightly longer than, that shown

* This description refers to scales on a wing mounted with the dorsal surface uppermost, in canada balsam, under slight pressure.

on the first vein. The fourth vein may have the dark area on the upper branch, or the second long pale area on the stem, interrupted.

Type ♂, Buta, November, 1922, Dr. R. Mouchet; co-type ♀ ♀ (3), one from Buta, one from Bambili, and one from Api, collected in November, 1922, by Dr. R. Mouchet. Other specimens from Bambili, 5 ♀ ♀, and Buta, 1 ♂, November, 1922, Dr. R. Mouchet; Basoko, Aruwimi, 18.2.1924, Service Médicale, 1 ♂, 2 ♀ ♀; districts de l'Equator et de l'Ubanguï, 18.9.1924, Dr. Trolli, 13 specimens; Kinshasa, Dr. Duren, 1922, 3 specimens.

The specimens were submitted for identification by Dr. G. Severin, of the Musée Royal d'Histoire Naturelle de Belgique, and Dr. H. Schouteden, of the Musée du Congo Belge.

Type ♂ and one co-type ♀ in the collection of the Musée Royal d'Histoire Naturelle de Belgique, the other co-type ♀ ♀ in the collection of the Liverpool School of Tropical Medicine.

The variety is named in honour of Dr. Mouchet, who has made extensive and valuable collections of Culicidae in the Belgian Congo.

This variety differs most obviously from typical *A. marshalli* Theo. in the absence of the interruption on the third large dark area of the first vein, and in the great length of the two distal white bands of the female palpi. Mr. F. W. Edwards, who very kindly compared co-type females of this variety with typical *A. marshalli*, informed me that the wing scales were shorter as well as broader than in the type form, agreeing with *A. domicolus* as regards their length, but that they were broader and denser than in this latter species. Mr. Edwards stated further that the variety resembled typical *A. marshalli* in having narrow hind tarsal rings, and differed from *A. domicolus* in this character.

THE IDENTITY OF THE RARER SCHISTOSOMES OF MAN AND THEIR INTERMEDIATE HOSTS

BY

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It is remarkable that at least four distinct types of Schistosome ova should occur in the urine of Natal patients, when only one type is known from the Far East and only two from Egypt. If the spindle-shaped ova were merely a variety of the ova of the *Schistosomum haematobium*, one would have expected them to occur in Egypt and if, as is thought, one type is that of *Schistosomum bovis*, one would have expected it to be more common in North Africa, in view of the heavy infestation of Sardinian cattle with this parasite and the relative immunity of South African cattle to Schistosome invasion.

In South Africa very little has been reported of the adult schistosomes and there is always an element of uncertainty where the diagnosis rests solely on the appearance of the ova that are detected in the urine of a patient.

A small ovum which is occasionally present in the urine of Natal patients has been regarded as that of *S. haematobium*, but its outline is identical with that of *S. bomfordi* Montgomery. There is certainly need for further research into the identity of those schistosomes which attack man in Africa. The subject is complicated by the difficulty that has been experienced in determining the usual intermediate host of the rarer schistosomes and because of the difficulty that therefore arises in rearing the adult parasites from the miracidia which escape from the ova in infested persons. Fig. 1 shows four different schistosome ova isolated from the urine of Natal schoolboys, as well as that of *Schistosomum japonicum* from the Far East and of two schistosomes from India.

In South Africa it is only rarely that schistosomes are found in fresh-water snails other than *Physopsis africana*. I have found schistosomes in both *Isidora globosa* Morelet and *Planorbis pfeifferi* Krauss at Laurenço Marques. Various *Isidorae* occur in South Africa; they are very commonly infested with amphistome cercariae. *Limnaea natalensis* is the common host of *Fasciola gigantica*. *Melanoides tuberculata* Müller is the only species with an operculated shell that I have found infested with cercariae; but J. D. F. Gilchrist has isolated cercariae from *Tomichia ventriculosa* (Sowerby) Reeve. *Ancylidae* harbour cercariae of various kinds and *Burnupia gordonensis* M. & P. is one of the commonest and largest species of this genus in South Africa.

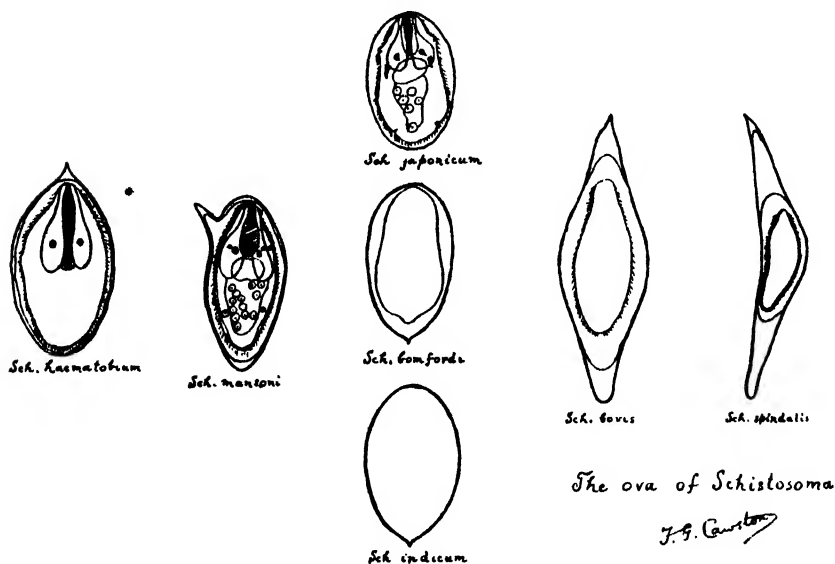


FIG. 1.

It is possible that a careful study of the radulae of intermediate hosts may assist in the determination of those species which resemble one another very closely. *Isidorae*, for instance, are notoriously variable and more than one species may occur in the same pool, each being represented by examples at various stages of growth. Although *Physae*, of which there are very few examples south of the Zambesi, might possibly be mistaken for *Isidorae* where the shell

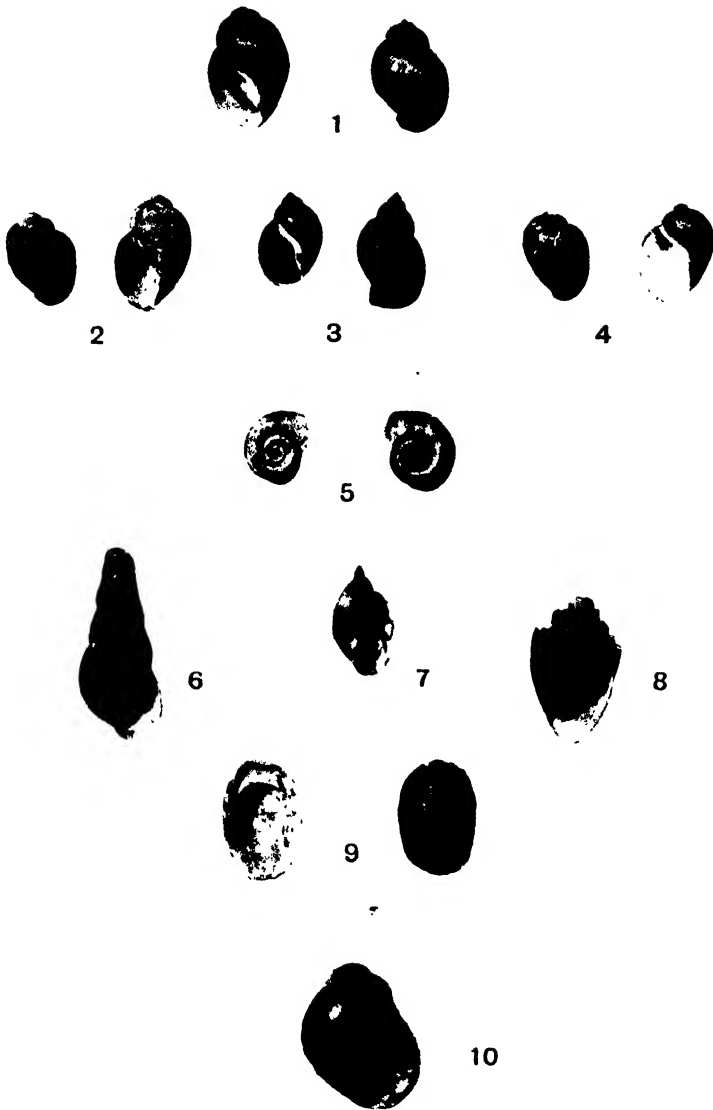


FIG. 2. 1.—*Physopsis africana* Krauss; 2.—*Isidora globosa* Morelet; 3.—*Isidora craveni* Ancey; 4.—*Isidora tropica* Krauss; 5.—*Planorbis pfeiffri* Krauss; 6.—*Melanoides tuberculata* Müller; 7.—*Limnaca natalensis* Krauss; 8.—*Tiara coacta* Meusch; 9.—*Septaria tessellata* Lamarck; 10.—*Theodoxus natalensis* Reeve.

alone is set aside for study, there is little danger of this mistake being made where the individual teeth of the two genera are examined. Although there is a good deal of variation in the appearance of the teeth in individual examples of the same species, even when taken from the same locality and grown under apparently identical conditions, yet a careful study of the teeth reveals the fact that this variation is almost confined to the CONES which grow from the CROWN of the tooth, so that the variation is not so great as at first sight appears.

THE INCUBATION PERIOD OF BENIGN TERTIAN MALARIA

BY

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The incubation-period of naturally-acquired benign tertian malaria is usually given as being from two to three weeks. Thus James (1920) states that the period is usually from 14 to 18 days, Stitt (1922) from 14 days, Acton cited by Castellani and Chambers (1919) 6 to 21 days, and Castellani and Chambers (1919) from 9 to 12 days. These authorities add that there are great variations in the length of the period outside the limits of the above figures. In addition, the cases of latent infection must also be remembered. In these cases several months, or even a year, may elapse since possible inoculation before the primary malarial attack develops. Often the onset is delayed until the patient undergoes some severe strain of either a mental or physical nature (James, 1920). The incubation-period of the naturally-acquired infection is thus seen to be extremely variable.

ARTIFICIALLY-INOCULATED MALARIA

(i) Mosquito-inoculations. Sir Ronald Ross (1911) gives details of cases inoculated artificially by means of infected mosquitos. The length of the incubation-period as measured by the first rise in temperature is recorded in nine instances and was found to vary from 10 to 14 to 25 days. In seven of these cases *Plasmodium vivax* was first demonstrated in from 16 to 30 days after infection.

In five general paralytics inoculated from mosquitos by Lieut.-Col. S. P. James the incubation-period varied from 11 to 16 days as regards the first definite malarial rise of temperature. In four of these cases the parasites were first found on the 17th and 18th days, three on the 17th and one on the 18th. Davidson (1925) found in a series of 23 cases that the period varied from 7 to 20 days.

In, therefore, a total of 37 cases the incubation-period, as measured by the first rise of temperature, varied from 7 to 25 days, and in 34 cases from 7 to 30 days as regards the first appearance of parasites, Davidson measuring the period by both methods.

(ii) Subcutaneous-inoculation. Most authorities give approximately corresponding lengths of time for the duration of the incubation-period when inoculation is practised subcutaneously. Thus Gerstmann (1924) states the period varies from 4 to 28 days, Scripture (1923) from 6 to 31 days, Donner (1925) from 5 to 21 days, Yorke and Macfie (1924) usually from 8 to 15 days, but with considerable variations, Worster-Drought and Beccle (1923) from 9 to 24 days and Nonne (1922) from 10 to 24 days. The table given by Pijper and Russell (1924) shows an incubation-period of from 9 to 18 days and that of McAlister (1924) from 9 to 32 days. In a series of 43 benign tertian malaria inoculations given by Grant and Silverston (1924) the first rise of temperature occurred from 1 to 18 days after inoculation, whereas parasites were first found from 6 to 22 days after. Korteweg (1924), in a series of 52 cases, found that parasites were first demonstrated in thick-films in from 5 to 21 days after inoculation.

Unfortunately, not all of the above authors state whether they define the termination of the incubation-period by the date upon which parasites were first found or by the date upon which the first increase of temperature occurred. It is, however, clear that the incubation period may be of many days' duration.

The incubation-period of a disease is that period which elapses between the admission to the body of the infecting organism and the first onset of symptoms. The latter may be subjective or objective (Gould, 1915). As the first appearance of symptoms may be objective in nature, the first day on which parasites are found in the peripheral blood-stream might be taken as the termination of the incubation-period. The disadvantage of this method, however, lies in the fact that there are so many factors to be taken into consideration when comparing the lengths of the incubation-period in different patients. At the commencement of an attack of malaria the parasites are usually comparatively few in number. In this case the day upon which the plasmodia are first found will depend upon: (a) whether thick or thin blood-films are used, (b) whether the whole or only a part of the film is examined and, if a part, which part (see below); and (c) the length of time each film is studied. In certain instances a fourth factor must be added: (d) the previous experience of the observer. If it were possible to

adopt general standard conditions, the method of measuring the incubation-period by the first appearance of the parasites would be of value.

With regard to the subjective symptoms it is clear that the length of the incubation-period cannot be determined by observing the occurrence of the first rigor, for many general paralytics inoculated with malaria do not shiver at all during their febrile treatment. The same applies to a subjective sensation of coldness, to sweating and to general enlargement of the spleen. The temperature, however, is raised during the initial malarial paroxysms, although, of course, parasites may be present during a relapse without fever occurring. But when the length of the incubation-period is under discussion malarial relapses do not enter into the subject. It should, however, be added that occasionally patients who have suffered from malaria previously may not develop febrile paroxysms but may show parasites for a few days. One such instance has occurred at Claybury Mental Hospital. In these cases, which are very rare, the method of recording the incubation-period by the first rise in temperature is not suitable. Now, non-inoculated general paralytics are subject, from time to time, to variations in temperature (Rudolf, 1925), and therefore an isolated elevation, or a succession of elevations, of temperature not immediately followed by typical malarial paroxysms or other definite signs of active malarial infection must not be taken as the termination of the incubation-period. As described by Korteweg (1924) and Rudolf (1924), some patients commence the attack of malaria by showing an irregularly moderately high temperature sometimes persisting for days, others by showing a series of elevations becoming progressively higher, and still others by a sudden very high elevation following a low, perhaps subnormal, temperature. Clearly, an initial rise of temperature to perhaps 100° F. in one case cannot be taken as the equivalent of a primary elevation to 105° F. in another case. To obviate this difficulty it is suggested that two rises of temperature be recorded to show the commencement of the malarial fever,—(a) the first elevation to 101° F. or over, and (b) the first to 103° F. or over. By adopting this method it is possible to tell at a glance whether a patient commenced his paroxysms suddenly or gradually. It will be observed that in this method increases of temperature under 101° F.

are not included. Now it is, of course, possible for a malarial paroxysm to show a rise of temperature of less than 101° F., but such a small rise of temperature would be extremely difficult to differentiate from an elevation accompanying the general paralysis. Rises of temperature above 101° F. are less common in untreated general paralytics.

In this connection the method adopted for recording the patient's temperature is important. From the time of inoculation the temperature should be recorded at least every four hours. Whenever it rises above normal it should be taken at least every hour, or even every ten minutes. The temperature varies so considerably within a short time that unless it is recorded very frequently the height of the fever might be missed.

The following table shows the length of the incubation-period as measured by the onset of the fever in the first 50 cases of general paralysis inoculated with *Plasmodium vivax* at Claybury Mental Hospital.

TABLE I

| First rise of temp. occurring | 101° F. TO 102.9° F. | | 103° F. OR OVER | |
|---|----------------------|-----------|-----------------|-----------|
| | No. of cases | Per cent. | No. of cases | Per cent. |
| Up to 10 days | 23 | 46 | 19 | 38 |
| From 11 to 20 days | 22 | 44 | 23 | 46 |
| From 21 to 30 days | 5 | 10 | 8 | 16 |

The above table shows that the greater number of cases show an incubation-period of less than twenty-one days.

With regard to the first appearance of parasites, Tables IV and V show the number of days after inoculation when parasites were first found. Table IV is adapted from Korteweg (1924). This observer used the thick-film method. Table V shows the first days on which parasites were found in cases treated at Claybury Mental Hospital. Korteweg does not state that he searched the thick-films for a definite length of time and similarly the thin-films of the Claybury series were not searched during a standard time.

TABLE II. Occurrence of first rise of temperature to 101° F.

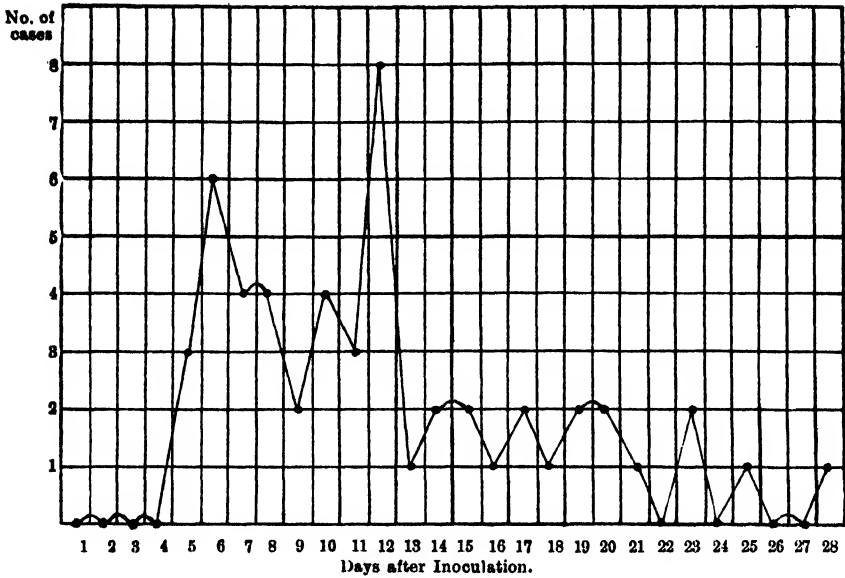


TABLE III. Occurrence of first rise of temperature to 103° F.

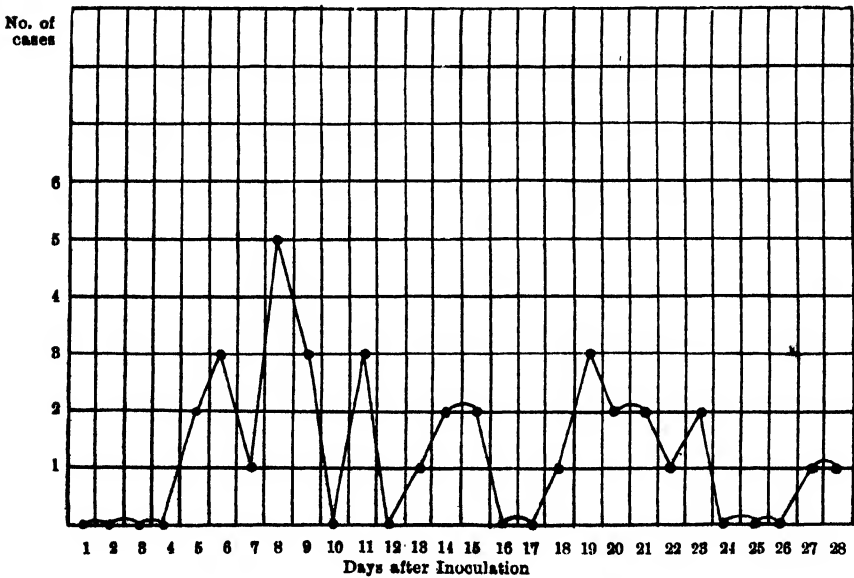


TABLE IV. First appearance of parasites in thick-films (adapted from Korteweg).

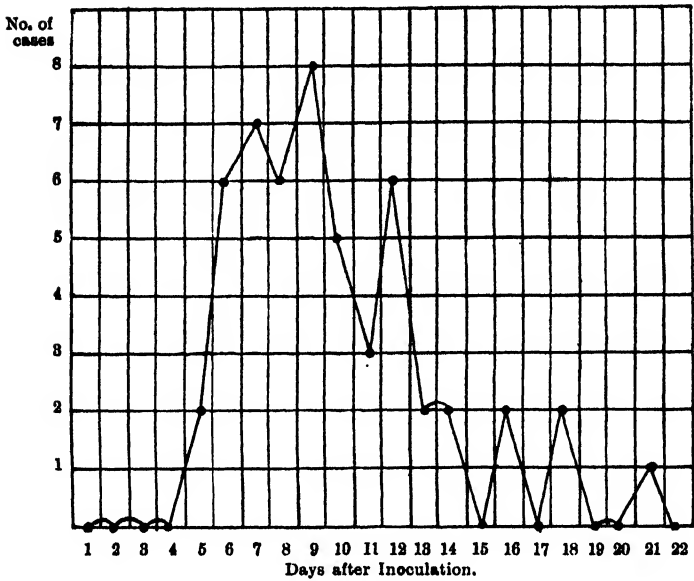
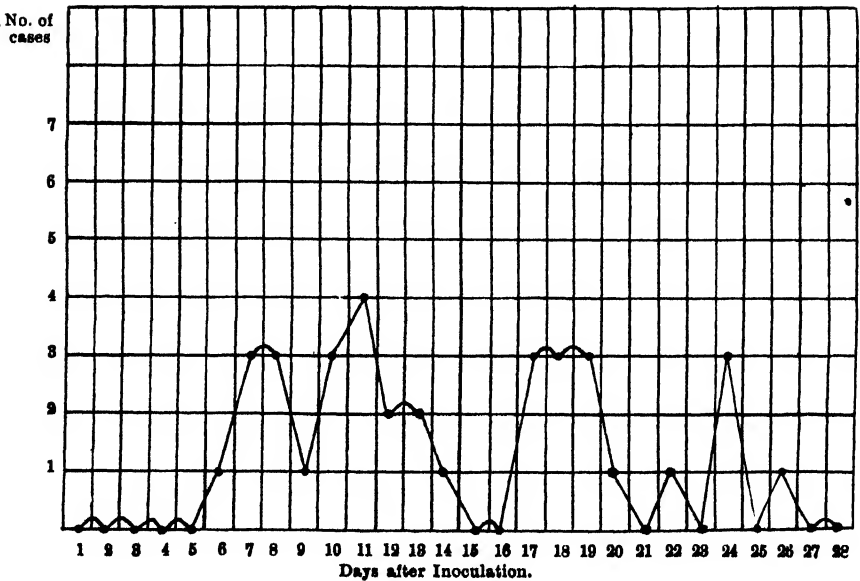


TABLE V. First appearance of parasites in thin-films.



On comparing Tables IV and V with Tables II and III it will be observed that the curves of the first rise of temperature to 101° F. and of the first time that parasites were found in thick-films are very similar. This is the more remarkable when it is remembered that the observations were made upon two series of cases treated with different strains of *P. vivax*. On comparing Table III with Table V a similarity between the curves will be again seen, both the curve of the first rise of temperature to 103° F. and the curve of the first time that parasites were found in thin-films being divided into three groups. The groups do not, however, correspond with regard to the periods in which they occur. The observations in Tables III and V were made on the same patients.

TABLES VI AND VII. Graphs of the lengths of the incubation-periods as measured by the first finding of parasites and by the first temperature-rises.

TABLE VI.

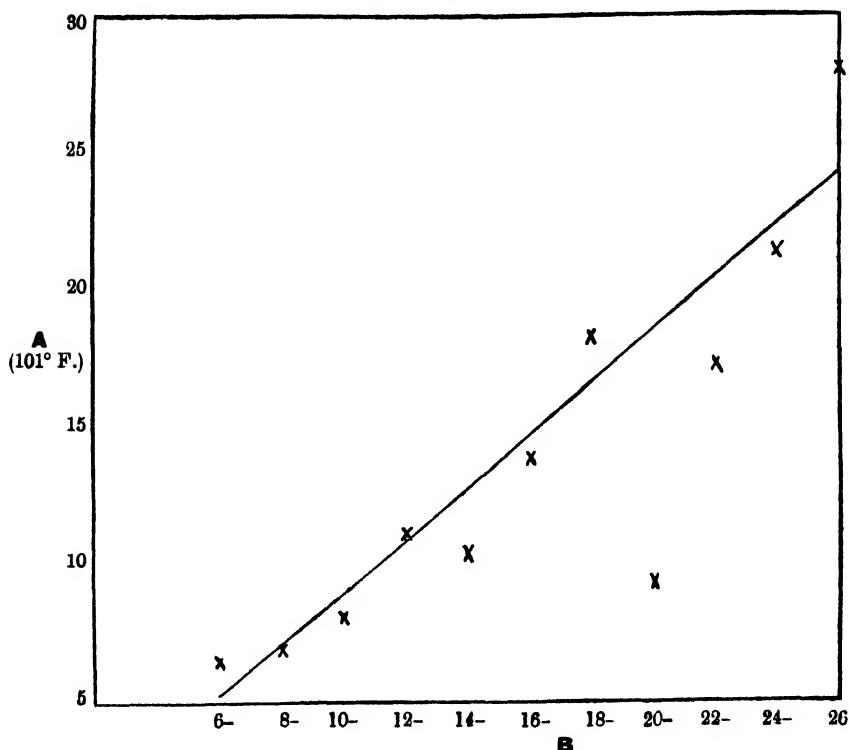
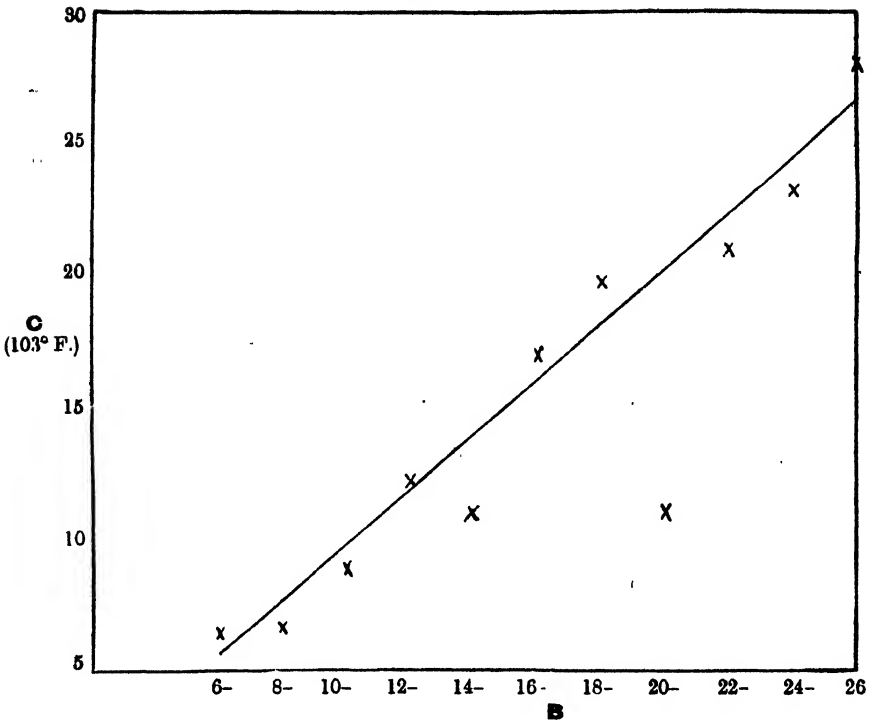


TABLE VII.



- A. Number of days to the first rise of temperature to 101° F. expressed as averages of each group of B.
- B. Number of days to the first finding of parasites. Cases arranged in two-day periods; e.g., patients in whom parasites were first found on the 6th and 7th days are grouped together in group 6-.
- C. Number of days to the first rise of temperature to 103° F. expressed as averages of each group of B.
- X = Average number of days before the first rise of temperature to 101° F. or 103° F., the diagonal lines are drawn to agree with the majority of the crosses.

If observations of the first time that parasites were found in thin-films, searched for varying periods, and of the first rises of temperature to 101° F. and to 103° F., in the same patients, are grouped, it will be seen that the means lie close to a straight line as shown in Tables VI and VII. These tables show that, in the same patients, there is a marked tendency for the parasites to be found for the first time when the first rise of temperature occurs. On working out the correlation-coefficients for the same variables a high correlation is observed. The coefficient for the first time that parasites were found and the first rise of temperature to 101° F. is + .9032, and that for the same first variable but the rise of

temperature to 103° F. is + .9091. There is, therefore, little difference between the correlation-coefficients when either the first rise to 101° F. or that to 103° F. is used.

The above confirms the observation that parasites are first found when the first rise of temperature occurs.

(iii) Intravenous inoculation. The duration of the incubation-period when this method is chosen would appear to be shorter than when the injection is made subcutaneously. Sir Ronald Ross (1911) gives details of six intravenous inoculations of benign tertian malaria. In these cases fever first appeared in from 3 to 12 days and parasites were first found in from 4 to 12 days after inoculation. Templeton (1924) states that in twenty cases of dementia praecox inoculated intravenously with 2 to 3 c.c. of malarial blood the temperature usually rose the day after inoculation. Macbride and Templeton (1924) found that pyrexia usually developed on the second or third day in a series of eighteen general paralytics. In both series of cases the temperature was, as a rule, irregular for a few days. Davidson (1925), in a series of sixteen general paralytics, found that the incubation-period varied from 4 to 19 days. The period was measured by the occurrence of fever and the first appearance of parasites.

Therefore, in 60 intravenous inoculations the incubation-period was found to vary from 1 to 19 days.

(iv) Intramuscular inoculation. Dr. D. R. Alexander has kindly supplied me with details of cases of general paralysis inoculated intramuscularly at Bexley Mental Hospital. The following table shows the length of the incubation-period as measured by the first rises of temperature. The strain of *P. vivax* utilised was the same as that used at Claybury Mental Hospital.

TABLE VIII

| First rise of temp. occurring | 101° F. TO 102.9° F. | | 103° F. OR OVER | |
|-------------------------------|----------------------|-----------|-----------------|-----------|
| | No. of cases | Per cent. | No. of cases | Per cent. |
| Up to 10 days | 9 | 60.0 | 8 | 53.3 |
| From 11 to 20 days | 6 | 40.0 | 6 | 40.0 |
| From 21 to 30 days | 0 | 0.0 | 1 | 6.7 |

On comparing the above table with Table I it will be observed that when the same strain of parasite is used there is a tendency for the incubation-period to be slightly shorter with intramuscular inoculation than with subcutaneous. On account of the relatively small number of cases, namely fifteen, in Table VIII it is probable that the differences between the lengths of the incubation-period with the two methods of inoculation are actually smaller than appears from the tables. This is in accordance with the findings of Davidson (1925). This observer noted that in a series of 13 cases the length of the incubation-period, as measured by the first fever and the first appearance of parasites, varied from 10 to 23 days, this approximating to the incubation-period when the subcutaneous route is adopted. In the two series of a total of 28 cases the incubation-period varied from 6 to 23 days as measured by the first occurrence of fever.

DOSAGE AND INCUBATION PERIOD

The usual dose of malaria-infected blood inoculated into general paralytics is from 1 to 5 c.c., although Pijper and Russell (1924) have used 10 c.c. There can, therefore, be great variations in the quantity injected. If one patient were inoculated with 2 c.c. of blood and a second with 4 c.c. it might be expected that the incubation-period of the former patient would be twice as long as that of the latter. But as the malarial parasite is the cause of the clinical signs of malaria it is clear that the volume of blood in itself can bear no relation to the incubation-period, but that the number of parasites present in the blood is the important factor. Therefore, in order to endeavour to determine whether there is any correlation between the number of parasites injected and the length of the incubation period it is necessary to know the actual, or the comparative, number of parasites in unit volume of blood. If the comparative number is chosen then the blood for inoculation must be drawn at one time from one patient, for the numbers of parasites vary in different cases, and also in the same case at different times.

The blood should then be divided and inoculated into the patients whose incubation-periods are to be compared. The blood must be well-shaken before each inoculation or the red cells containing the parasites will sink to the bottom and the patients will not receive the correct number of red cells according to the volume of blood injected. For the same reason no more than the exact quantity of blood required for each injection must be sucked into the syringe. If more than required is in the syringe, the first patient to be inoculated may receive too few or too many erythrocytes per cubic centimetre according to whether the needle of the syringe is held pointing upwards or downwards.

The above comparative method has been used in the study of a series of cases inoculated subcutaneously with benign tertian malaria at Claybury Mental Hospital. The following method was that adopted for determining the first appearance of the parasites. Thin-films were examined daily, commencing seven days after inoculation, except in certain cases in whom the rises of temperature started before that date. After the first appearance of parasites in the films had been found in this manner, more accurate observations were made. The films taken on the day previous to the first appearance of the parasites were each examined during a standard time of thirty minutes. Particular attention was paid to the edges and to the 'tags' of blood at the end of the film as parasites are often found in greater numbers in these situations than in the remainder of the film. Table IX shows the results obtained. The patients bracketed together were inoculated from the same patient. The total quantity of blood required was withdrawn, divided into the necessary quantities, and injected into the general paralytics to be treated. The relationship between the quantity of blood, and therefore the comparative number of parasites inoculated, and the length of the incubation-period can therefore be studied in each series of cases bracketed together. The table shows the duration of the incubation-period as measured by the date on which the first rise of temperature to 101° F. and to 103° F. occurred.

TABLE IX

| Series No. | Patients No. | Sex | Dose in c.cs. | IN DAYS | | |
|------------|--------------|-----|---------------|-----------------------|----------------------|----------------------|
| | | | | Parasites first found | 1st Temp. to 101° F. | 1st Temp. to 103° F. |
| 1 | 1 | M | 2 | 17 | 17 | 17 |
| | 1A | M | 2 | 13 | 9 | 9 |
| 2 | 2 | M | 2 | 14 | 11 | 13 |
| | 2A | M | 2 | 16 | 19 | 19 |
| 3 | 3 | M | 1.5 | 7 | 7 | 10 |
| | 3A | F | 1.5 | 5 | 7 | 8 |
| 4 | 4 | M | 3 | 19 | 21 | 22 |
| | 4A | F | 3 | 19 | 18 | 18 |
| 5 | 5 | M | 5 | 12 | 8 | 9 |
| | 5A | F | 5 | 26 | 26 | 29 |
| 6 | 6 | M | 5 | 6 | 5 | 6 |
| | 6A | F | 4.5 | 8 | 6 | 6 |
| 7 | 7 | M | 3 | 18 | 20 | 20 |
| | 7A | F | 5 | 11 | 6 | 8 |
| 8 | 8 | M | 2.1 | 9 | 8 | 9 |
| | 8A | M | 4 | 6 | 7 | 8 |
| 9 | 9 | M | 2 | 11 | 6 | 8 |
| | 9A | M | 3 | 11 | 6 | 6 |
| | 9B | M | 4 | 7 | 6 | 11 |
| | 9C | M | 5 | 7 | 8 | 10 |
| 10 | 10 | M | 2 | 21 | 17 | 19 |
| | 10A | F | 4 | 16 | 15 | 15 |
| | 10B | M | 8 | 13 | 12 | 14 |

The above table may be divided into two groups: the first consists of the series Nos. 1 to 5, the patients in each series being given the same number of parasites; the second consists of the series Nos. 6 to 10, the patients in each series being given different

numbers of parasites. Series Nos. 1 to 4 show that when there is the same dosage of parasites the length of the incubation-period is somewhat similar in each series. In series Nos. 1, 2, and 4, it is more nearly similar when it is measured by the time that parasites were first found than by the first rises of temperature. In series No. 5 there is a marked difference between the length of the period in the two patients. Patient 5A had been inoculated previously with malaria but had not 'taken.' The resistance of this patient was presumably high. After the second inoculation, however, all the parasites could not have been destroyed but, if a large number were, the same effect would be produced as if a small number had been injected.

The later series of the table, Nos. 6 to 10, show the effect of inoculating different numbers of parasites. It will be observed that, in each series, the cases that received the smaller dose gave the longer incubation-period as measured by the first appearance of parasites. This was also found to hold when the length of the incubation-period is measured by the first rise of temperature except in one series, No. 9. In this series the patients that received the greatest number of parasites showed the longest incubation-periods as regards the first rise of temperature, but the shortest as regards the first appearance of parasites. It will also be observed that the incubation-periods measured by the first time that parasites were found agree more nearly with the dosage, in an inverse relationship, than do the same periods when measured by the first rise of temperature.

SUMMARY

(i) The incubation-period of the naturally-acquired benign tertian malaria is given by most authorities as being from 6 to 21 days with, however, wide variations.

(ii) In 34 cases (chiefly from the literature) inoculated by means of anopheline mosquitos, the incubation-period varied from 7 to 30 days as measured by the first date on which parasites were found, and, in 37 cases, from 7 to 25 days as measured by the first rise of temperature.

(iii) Subcutaneous inoculation of malarial blood gives, according to a number of writers, an incubation-period of from 1 to 32 days. A series of 50 general paralytics inoculated subcutaneously with *Plasmodium vivax* showed that 90 per cent. gave a rise of temperature of from 101° F. to 102.9° F. in less than 21 days after inoculation and 46 per cent. in less than 10 days. The first rise of temperature over 103° F. occurred within 21 days in 84 per cent. and within 10 days in 38 per cent.

(iv) Following subcutaneous inoculation there is a well-marked correlation between the first rises of temperature to 101° F. and to 103° F. and the first finding of parasites in thin-films. The frequency-curves of the first finding of parasites in thick-films and of the first rise of temperature to 101° F. are very similar, although the observations were made upon two different series of cases inoculated with two different strains of parasite. Curves of the first finding of parasites in thin-films and the first rise of temperature to 103° F., in the same series of cases, are also somewhat similar.

(v) Intravenous inoculation of malarial blood gave an incubation-period of from 1 to 19 days in a series of 60 cases collected from the literature.

(vi) In a series of 28 cases inoculated intramuscularly at Bexley and Winwick Mental Hospitals the incubation-period varied from 6 to 23 days.

(vii) In 10 series of cases injected subcutaneously with malarial blood it was found (a) that when similar numbers of parasites were injected the incubation-periods were of somewhat similar lengths, and (b) that when different numbers of parasites were injected the incubation-periods showed a marked tendency to be shortest when the dosage of parasites was the greatest. In one series of four cases this relationship did not hold as regards the first rises of temperature but only as regards the first dates on which parasites were found. In most series the length of the incubation-period as measured by the first time that parasites were found, under standard conditions, corresponded more nearly to the dosage than did the length of the period as measured by the first rises of temperature.

I have again to thank Lieut.-Col. S. P. James for his kind assistance. My thanks are due to Drs. G. F. Barham and G. Clarke, Medical Superintendents of Claybury and Bexley Mental Hospitals,

for permission to publish records from the two Institutions, and to Dr. M. Greenwood for his kindly help.

I am indebted to Dr. D. Firth, of King's College Hospital, and to Dr. F. Kiddle, of Severalls Mental Hospital, for details of cases.

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THE MODE OF ACTION OF BAYER '205' ON TRYPANOSOMES

BY

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Since its introduction the drug Bayer '205' has excited the interest of various workers engaged in the study of trypanosomes or of general chemotherapeutic problems. This interest is due partly to its active trypanocidal properties and partly to the peculiarity of its behaviour *in vivo* and *in vitro*. *In vivo* it excites a profound reaction, modifying the coagulability of the blood (Steppuhn, Zeiss u. Brychonenko, 1923) injuring the red blood cells (Sei, 1923), stimulating a lymphocytic response (Kligler & Weitzman, 1924), and, in larger doses, producing marked toxic effect on the kidneys (Duncan and Manson-Bahr, 1924). Unlike most other drugs it is retained in the body in active form for weeks after the injection (Mayer u. Zeiss, 1920, and Ruppert, 1923). Another striking peculiarity is the apparent difference in its trypanocidal power *in vivo* and *in vitro*; *in vivo* it is active in small doses, while *in vitro* it is apparently ineffective.

All those who have experimented with the drug agree as to its profound effect on the parasites in animals; but there is a considerable amount of controversy as to the mode of action on the trypanosomes. Morphological studies by Steffan (1922) and by Hesselbach (1922) indicated a direct effect on the cell protoplasm, and observations by Mayer and Zeiss (1920) and Shintake (1923) suggested an influence on the process of division. The work by Haendel and Yotten (1920) showed that there is a direct combination between the trypanosomes and the drug and that the latter cannot be released by washing. Ruppert (1923), on the other hand, concluded from his experiments that Bayer in its active form is not fixed *in vitro* although it does exert some effect, and that the action of the drug is indirect.

The nature of the action of the drug is of more than theoretical importance. If its effect is really an indirect one, it follows that the usual *in vitro* estimation of the parasitocidal property of a drug is of little value as an indication of the behaviour of the drug in the animal body. It seemed of interest, therefore, to investigate further the effect of the drug on the trypanosomes *in vitro* and to ascertain whether there is any relation between its effect *in vitro* and *in vivo*.

As our experiments were drawing to a close Nauck (1925) published a paper on the same subject. This article does not, therefore, present any new information, but our experiments serve to supplement as well as confirm Nauck's findings.

Nauck worked with a strain of nagana trypanosome and used mice as his culture medium; infecting rabbits, treating them with Bayer, and then, at varying intervals after treatment, infecting mice with the blood of the treated rabbit. Nauck used large doses of Bayer and carried on most of his experiments *in vivo*.

We used a strain of *Tr. evansi* and our procedure differed from Nauck's in that the exposure of the trypanosomes to the drug were made *in vitro* and only the effect on the organisms tested by inoculation into animals to determine loss of virulence. Our experiments were also designed to obtain an approximate quantitative comparison of the trypanocidal power of the drug *in vitro* and *in vivo*.

The following is a brief presentation of the principal experiments bearing on this question.

The object of the first series of experiments was to ascertain whether exposure to the drug in any way affected the virulence of the trypanosomes. After exposure of the organisms for varying lengths of times in varying dilutions of the drug, the trypanosomes were sedimented and injected into guinea-pigs or rabbits. Adequate controls were always made.

EXPERIMENT I.—The first experiment consisted in exposing suspensions of trypanosomes in serum to which varying dilutions of Bayer were added. The suspension was kept three hours at 25° C. centrifugalized, the supernatant solution containing the drug was decanted, and the sediment inoculated into rabbits and guinea-pigs. The details of this experiment are given in the protocols.

Protocol a, Experiment 1. Date 23.9.24.

4 c.c. blood from a guinea-pig containing 4 trypanosomes per microscopic field, defibrinated; 1 c.c. saline added; centrifuged at 700 revolutions for 5 minutes; opalescent fluid withdrawn; divided into 3 parts; to 1 part added Bayer in concentration 1/200; to 1 part 1/400; 1 part-control; kept 3 hours at 25° C. (incubator); centrifuged; fluid decanted, sediment shaken in 1 c.c. saline, injected half into a rabbit (R.) and half into a guinea-pig (G.p.).

R. 12. Injected tryps. in Bayer 1/200; after 5 days positive.

R. 13. Tryps. in Bayer 1/400; after 9 days positive.

R. Control. After 9 days positive.

G.p. 38. Tryps. in Bayer 1/200, after 5 days positive.

G.p. 39. Tryps. in Bayer 1/400, after 10 days positive.

G.p. Control. After 9 days positive.

Protocol b, Experiment 1. Date, 2.10.24.

Guinea-pig punctured; numerous parasites; 3½ c.c. blood taken; defibrinated by means of beads; 1 c.c. saline added, centrifuged. To the plasma added Bayer to concent. 1/100, 1/200, 1/400; ½ c.c. quantum taken, kept 3 hours, centrifuged, serum decanted; to sediment added ½ c.c. saline and 0.2 c.c. injected into each animal.

R. 15. Bayer 1/100; negative, observed 37 days; 10.11.24 injected 10 c.c. oil; observed 18 days; negative; superinfected; positive after 7 days.

R. 16. 1/200; died after 5 days; intercurrent infection.

R. 16a. 1/400; died after 5 days; intercurrent infection.

R. 17. Control. Died after 5 days; intercurrent infection.

G.p. 41. Bayer 1/100; observed 37 days; results negative. 10.1.24 injected 4 c.c. oil, observed 18 days; negative. 18.11.24 superinfected; positive after 4 days.

G.p. 42. Bayer 1/200; negative; history same as g.p. 41.

G.p. 43. Bayer 1/400; positive after 14 days.

G.p. Control. Positive after 10 days.

5.10.24 preparation of material the same as that of 2.10.24 and injected again into

R. 18. 1/200.

R. 19. 1/400. To replace R. 16 and R. 16a.

R. 18. 1/200, negative; observed 35 days. 10.1.25, 10 c.c. oil; observed 18 days; negative; superinfected; positive after 5 days.

R. 19. 1/400 positive after 14 days.

It appears that contact of trypanosomes for three hours with a 1:100 dilution of the drug is sufficient to destroy their virulence; a 1:200 dilution gave variable results; in one experiment the organisms were still infective, in the other not; a three-hour exposure to 1:400 dilution did not completely destroy the virulence, but the incubation period was prolonged, indicating a certain degree of injury.

EXPERIMENT 2.—This experiment was similar to No. 1, except that the exposure was for twenty-four hours. The results as shown in the protocol were negative, even in a dilution of 1 : 400.

Protocol a, Experiment 2. Date, 13.10.24.

Two guinea-pigs punctured; $3\frac{1}{2}$ c.c. and $2\frac{1}{2}$ c.c. blood taken; positive 7 per field; defibrinated; 2 c.c. saline added; centrifuged; supernatant fluid containing tryps. withdrawn; Bayer added to dilution 1/200, 1/400 in quant. of $\frac{1}{2}$ c.c.; fluid left for control. After 24 hours suspensions examined; tryps. alive; sluggish motion; tubes centrifuged; clear fluid decanted saline added to sediment and injected with glass capillaries intraperitoneally.

- R. 25. 1/200; negative; observed 30 days. 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; 11 days incubation.
 R. 24. 1/400; negative; observed 30 days; 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; incubation 9 days.
 R. 25. Control; positive after 6 days.

Protocol b, Experiment 2. 12.1.24.

Two guinea-pigs bled; $3\frac{1}{2}$ c.c. blood; defibrinated; centrifuged slow speed until fluid opalescent.

| | | |
|---|---|---|
| 0.25 c.c. susp. of tryps. 0.25 c.c. 1/100 Bayer | 0.25 c.c. susp. of tryps. 0.25 c.c. 1/200 Bayer | 0.25 c.c. susp. of tryps. 0.25 c.c. saline |
| 0.50 c.c. 1/200 After 24 hours at 25° C.—1/200; Alive; peristaltic movements of the undulating membrane. Motion sluggish. | 0.50 c.c. 1/400 Control for motility; 1/400; Active movement. | Control Control; Active movement. |

Tubes centrifuged 10 minutes at highest speed; clear serum decanted; added $\frac{1}{2}$ c.c. saline to each tube; injected 0.2 c.c. into each animal.

- R. 27. 1/200 negative; observed 44 days; superinfected; positive; 5 days incubation.
 R. 28. 1/400 negative; after 44 days superinfected; positive after 5 days.
 R. 26. Control; positive after 5 days.

EXPERIMENT 3.—This was a repetition of Experiment 1, namely, a three-hour exposure, but a smaller number of trypanosomes was injected; the results showed that even an exposure of three hours to 1 : 400 dilution of the drug renders the organisms non-infective.

G.p. 34. 3 c.c. (tryps. 1 per field) taken; defibrinated; added 1 c.c. saline; centrifuged; dilutions to 1/200, 1/400 Bayer made as in experiment date 12.1.25; kept 3.15 hours in incubator at 25° C.; centrifuged, clear fluid decanted; to sediment added $\frac{1}{2}$ c.c. saline; shaken; 0.2 c.c. injected into each animal.

- R. 34. 1/200 negative; observed 19 days; superinfected 1.3.25; positive after 7 days.
 R. 35. 1/400; negative; observed 19 days; 1.3.25 superinfected; positive; incubation 5 days.
 R. Control. Positive; incubation 5 days.

EXPERIMENT 4.—This experiment was a repetition of Experiment 2 (twenty-four hours exposure), except that higher dilutions of the drug were used (800 and 1,600). The results indicated that even as low a concentration of the drug as 1 : 1600 is sufficient to destroy the virulence of the organisms. The control trypanosomes in each case were put through the same manipulations as the drug-exposed organisms, so that the possibility of loss of virulence through mechanical injury was eliminated.

Protocol Experiment 4. 7.2.25.

Two guinea-pigs bled $2\frac{1}{2}$ c.c. ; tryps. 3 per field ; defibrinated ; added 1 c.c. saline ; centrifuged at 750 revolutions ; opalescent fluid still containing few r.b.c. used. Dilutions with Bayer made ; opalescent fluid taken ; 0.9 c.c., $\frac{1}{2}$ c.c., $\frac{1}{2}$, etc., to the first tube added ; 0.1 c.c. 10% Bayer ; dilution obtained 1/100 ; $\frac{1}{2}$ c.c. transferred to second tube ; etc. Final dilutions 1/100, 1/200, 1/400, 1/800, 1/1600 ; tubes left for 24 hours in the incubator at 25° C. After 24 hours examined ; 1/100—slight undulant movement ; 1/200 sluggish movement
1/400 active movement
1/800 active movement
1/1600 active movement
Control active movement.

Tubes 1/800, 1/1600 and control ; centrifuged ; clear fluid decanted ; sediment diluted in $\frac{1}{2}$ c.c. saline ; tryps. still active ; injected 1/800 into rabbit 48 ; 1/1600 into rabbit 49 ; control into rabbit 50.

R. 48. Observed 30 days ; negative.

R. 49. Observed 30 days ; negative.

R. Control. Positive after 6 days ; heavy infection.

This series of experiments showed that Bayer ' 205 ' has a marked effect on trypanosomes *in vitro*. Ordinarily this effect is overlooked because the result is judged by the motility of organisms. The motility is not, however, an index of protoplasmic injury and the principal effect of the drug lies in a lowering or destruction of virulence due presumably to cell injury.

On the basis of these experiments made *in vitro*, it appears that the action of the drug *in vivo* is also direct and that the therapeutic as well as prophylactic action of the drug depends on the concentration of the drug in the body and the rate of elimination by a given host.

Previous therapeutic experiments showed clearly that the drug is active in certain proportional doses, at least in so far as rabbits and guinea-pigs are concerned. A dose of 0.1 gm. per kilo cured all animals ; 0.05 gms. per kilo gave about 80 per cent. cures, while 0.005 gms. per kilo was not effective.

This relation of dose to effect is further illustrated by the following experiment. The purpose of this experiment was to see whether an infection can be aborted by a dose of Bayer smaller than the therapeutic dose. As is seen from the protocol below, the abortive dose is the same as the therapeutic dose ; 0.05 gm. per kilo aborted the infection, while 0.005 gm. did not.

Protocol Experiment 5. 13.12.24.

Two rabbits of same weight infected 13.12.24 ; on 16.12.24 rabbit 015 given 0.005 gm. Bayer per kilo and rabbit 016 was given 0.05 gm. per kilo. R. 015, 28.12.24, blood positive. R. 016, blood negative ; observed 32 days and continued negative.

The next series of experiments dealt with the prophylactic property of the drug. The object was to ascertain whether there was any relation between dose and the duration of protection. In other words, we tried to determine the relation between concentration of the drug and prevention of infection.

Experiment 6. Three rabbits injected with different doses of Bayer and at varying intervals ; after treatment the animals were infected.

R. 017. 0.05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative.

R. 018. 0.05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative.

Infected again after three months ; positive, after incubation of 15 days.

R. 019. Injected 0.1 gm. Bayer per kilo ; one month later infected ; negative ; reinfected after another month ; trypanosomes appeared in the circulation after a delay of three weeks.

This experiment indicates that Bayer apparently confers protection only so long as the drug remains in the body in a concentration sufficient to affect the parasites. This relation between concentration of drug and protection is further emphasised by the subsequent experiment.

EXPERIMENT 7.—The object of this experiment was to determine whether the minimal protective dose corresponds to the minimal therapeutic dose. In this experiment the infection was given within a week or two after the injection of the drug. It is evident from the results that a dose of 0.005 gm. per kilo failed to give any protection just as this dose is devoid of any therapeutic effect.

R. 022. Given 0.005 gms. Bayer ; two weeks later infected ; positive after ten days.

R. 023. Given 0.01 gm. Bayer per kilo and infection followed 8 days later ; results negative ; animal observed two months.

R. 024. Given 0.005 gms. Bayer per kilo ; infected 10 days later ; positive after 7 days.

ANALYSIS OF EXPERIMENTS.—The various experiments described above bring out two facts. First that contrary to our previous belief that Bayer exerts little trypanocidal action *in vitro*, it appears that the drug has a marked effect on the cell so that a dilution of 1 : 1600 is sufficient to destroy the virulence of the organisms. The other fact is that, in rabbits at least, the therapeutic, abortive and prophylactic doses are similar.

It is difficult to make comparisons between *in vitro* and *in vivo* effect, because it is not possible to determine the amount of drug which remains in circulation. The work of Mayer and Zeiss indicates that the drug is bound in the blood stream, by the serum, and is thus retained for many weeks. If the weight of the blood is accepted as approximately 1/15 the total body weight, it is possible to make a rough estimate of the effective dilution of the drug in the circulation. Our experiments show that doses of 0.005 gm. per kilo, or a dilution of 1 : 3000, fails either to protect or cure an animal while 0.01 gms. per kilo, or a dilution of 1 : 1500, is effective in a proportion of cases. Even if we assume that only 50 per cent. of the drug is bound in the serum, the effective doses *in vivo* correspond fairly well with those *in vitro*. A still further correspondence is the fact that when small doses of the drug are given the trypanosomes disappear from the circulation only sixteen to eighteen hours after treatment.

The rational conclusion then is that the therapeutic property of Bayer '205' is due to a direct injury to the trypanosomes which renders them avirulent for the host and thus readily destroyed and eliminated. The difference observed in different hosts are probably due to the rate of elimination of the drug, or in other words, to the residual concentration of the drug in the circulation.

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ON A NEW CESTODE FROM NIGERIA

BY

T. SOUTHWELL.

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A single specimen of a cestode worm from the small intestine of a 'large grey eagle' was obtained by Dr. Ll. Lloyd at Sherifun, Northern Nigeria, 24.12.24. The species is new and is described as follows :

LATERIPORUS FUHRMANNI, n.sp. (figs. 1-4)

EXTERNAL ANATOMY:—The worm was fragmented but apparently measured about 20 cms. in length; its maximum breadth is 1 mm. It is composed of a very large number of segments, the posterior margins of which are imbricated; the most posterior segments are gravid, somewhat bell-shaped and as long as broad. The genital pores are unilateral and are situated just in front of the middle of the lateral margin.

Head. The head is somewhat oval and measures about 450μ by 330μ . It is armed with a single crown of about fourteen hooks, each of which measures about 31μ in length.



FIG. 1. *Lateriporus fuhrmanni* n.sp. Head. $\times 75$.

Neck. A neck is present but, owing to the fact that the worm was fragmented, its length could not be determined.

INTERNAL ANATOMY:—As only a single worm was available, details relating to the muscular, nervous and excretory systems were not investigated.

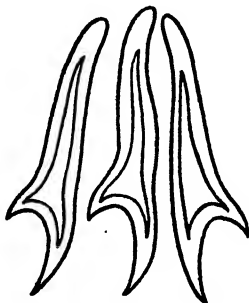


FIG. 2. *Lateriporus fuhrmanni* n.sp. Hooks. $\times 1125$.

Testes. There are about twenty-five testes situated posteriorly, behind, and lateral to the ovary. In full development they have a diameter of about 50μ .

Vas deferens. The cirrus pouch is situated anterior to the vagina, and it varies a little in shape; usually it is a cylindrical organ extending in the median direction to the excretory vessel; its median extremity appears glandular. The vas deferens is a long coiled tube, situated in front of the ovary and surrounded with a mass of prostatic glands.

Ovary. This is a bilobed organ composed of large acini situated in front of the testes.

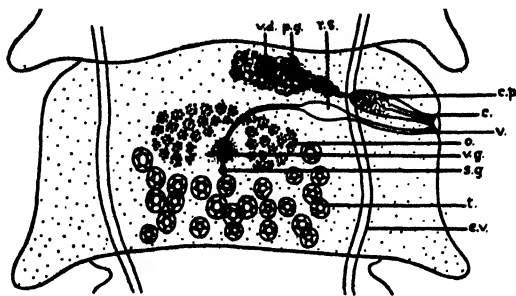


FIG. 3. *Lateriporus fuhrmanni* n.sp. Mature segment. c.—cirrus; c.p.—cirrus pouch; t.—testes; v.d.—vas deferens; p.g.—prostatic glands; r.s.—receptaculum seminis; v.—vagina; o.—ovary; v.g.—vitelline glands; s.g.—shell gland; e.v.—excretory vessels. $\times 75$.

Vagina. The vagina runs posterior to the cirrus pouch and, immediately median to the excretory vessels, it dilates into a large pear-shaped receptaculum seminis.

The vitelline and shell glands lie immediately behind the ovary, the shell gland being very small.

Uterus. The uterus consists of a simple sac which completely fills the segment.

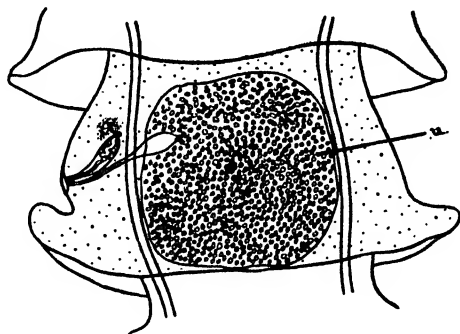


FIG. 4. *Lateriporus fuhrmanni* n.sp. Gravid segment. u.—uterus.

Eggs. No fully mature eggs were seen.

DIAGNOSIS. The single crown of hooks on the head, the unilateral pores, the posterior testes and the sac-like uterus place this worm in the genus *Lateriporus* Fuhrmann 1907. Six species of this genus are known.

The following table shows how *L. fuhrmanni* differs from the other species of the same genus, viz., principally in the size of the hook.

| | Length of worm | No. of hooks | Size of hooks | No. of testes |
|--------------------------------------|---------------------|--------------|---------------|---------------|
| <i>cylindrica</i> (Clerc, 1902) ... | 25 mm. | 16 | 200-216 μ | 15 |
| <i>teres</i> (Krabbe, 1869) ... | 42-60 mm. | 12-16 | 150-170 | 30 |
| <i>biuterinus</i> Fuhrmann, 1908 ... | 300 mm. | 16 | 120 μ | 16-18 |
| <i>spinosus</i> Fuhrmann, 1908 ... | 40 mm. | 22 | 50 μ | 6 (?) |
| <i>propeteres</i> Fuhrmann, 1907 ... | several centimetres | 16 | 120 μ | about 12 |
| <i>geographicus</i> Cooper, 1921 ... | 172 mm. | ? | ? | 15-20 |
| <i>fuhrmanni</i> n.sp. ... | about 200 | about 14 | 31 μ | 25 |

The type specimen is in the collection of the Liverpool School of Tropical Medicine.

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SOME CHARACTERISTICS OF THE FIRST STAGE LARVA OF *DERMATOBIA HOMINIS* GMELIN

BY

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AND

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(Received for publication 8 June, 1925)

PLATES IV AND V.

In Central and South America there is an Oestrid Fly, *Dermatobia hominis*, whose larva is the cause of cutaneous Myiasis in man and animals. These larvae are able to penetrate the unbroken skin, and there, in the course of development to maturity, give rise to tumours similar to those produced by the larvae of the Warble Flies (*Hypoderma* spp.) of Europe and North America. They are a source of considerable loss to cattle-owners, since the hides, riddled by the holes left on the emergence of the fully-grown larvae, are often valueless. According to Da Matta (1920), the proportion of hides thus damaged may be from 5 per cent. to as much as 70 per cent. The larvae are also indirectly responsible for the death of many animals, especially calves, since the tumours caused by them are liable to secondary infection from other myiasis-producing flies, whose larvae are unable themselves to pierce the unbroken skin. There is one fly of this latter type, the 'Screw-worm' (*Chrysomya macellaria*), which is very abundant in the Neotropical Regions and which in this way does a great deal of damage. Lastly, the *Dermatobia* larvae are the cause of much pain and inconvenience to man when passing through infected regions, and even give rise to serious illness if present in large numbers.

This Oestrid Fly differs from all other members of its class in that, instead of laying eggs or larvae directly on the hair or skin of the host, it lays batches of its eggs on the bodies of other insects, chiefly mosquitos. The larva of *Dermatobia* does not, apparently, leave the egg until the mosquito alights on a warm-blooded animal

to take a meal. Thus it would seem that the larva must be highly sensitive to a slight rise in temperature, and that the emergence from the egg must of necessity take place during the comparatively brief period in which the mosquito feeds. There is evidence to show that the larva, if unable to emerge completely from the egg during the time the mosquito is feeding, may withdraw itself into the egg and there wait until the mosquito visits another animal. This may occur several times, the larvae being capable of remaining alive for twenty days before reaching a host.

We are indebted to Dr. Nunez Tovar, who himself has done much to elucidate the remarkable life-history of the *Dermatobia*, for his gift of material which has enabled us to study these young larvae which penetrate the skin of their hosts. Before we present a detailed description of its characteristics, it has been thought well to give a short account of the life-history of this fly, as some interesting work has been done since the last comprehensive account in English was written by Sambon in 1915.

THE DISTRIBUTION OF THE FLY

According to Neiva and Gomes (1917), *Dermatobia hominis* occurs in Central and South America from Mexico to the Argentine. It appears to be absent from the United States, for, although cases infested with the larvae have been recorded from that country, it has always been found that the larvae were acquired in Central or South America. The fly seems to need a warm temperature, a certain degree of humidity, and forest country.

HOSTS OF THE FLY

The occurrence of larvae in the skin of man and various animals has long been known. The domestic animals in which they have been found are, in order of importance :—Cattle, dogs (especially hunting dogs), pigs, goats, turkeys, and, rarely, mules. There appears to be some doubt as to whether they occur in sheep, donkeys and horses. It is, in fact, sometimes stated that horses are not infested, but Neiva and Gomes (1917) give one record of the finding of larvae in a horse. The larvae have also been found in the

following wild animals :—Monkeys (whence the name ' Ver Macaque,' frequently given to the larva), jaguar, tapir, coati, agouti, deer (rarely), squirrels, and even birds, e.g., toucans and the ant-thrush (*Formicarius* sp.).

DISPOSAL OF THE EGGS

Although the existence of these larvae has been known for so long, there has been considerable doubt and uncertainty as to the exact way in which they reach the skin of their hosts.

Morales (1911), in Guatemala, was the first to publish a statement to the effect that the eggs were carried, firmly attached to the abdomen of a mosquito, *Psorophora* (then *Janthinosoma*) *lutzi*. From such eggs Morales obtained a larva, which produced in man characteristic tumours, and which presented all the characters of a *Dermatobia* larva. Tovar, in Venezuela, made similar observations two months earlier, but these were not published until 1913, in an article by Gonzales Rincones in the newspaper 'El Universal' of Caracas.

These observations have since been confirmed by other observers, different flies being found to be *involuntary carriers* of the eggs in different parts of the country. Specimens of *Dermatobia* astride other flies have been caught, and even observed in the act of siezing the flies. According to Neiva and Gomes (1917), the adult *Dermatobia* frequent horses and other animals, and sieze flies which come either to suck blood or to feed upon sweat.

The final piece of evidence, the observation of the act of deposition of the ova by *Dermatobia*, has also been recorded. Neiva and Gomes (*loc. cit.*) enclosed adult females with various flies and found that eggs were laid on *Musca domestica*, *Stomoxys calcitrans*, and also on some Sylvan Muscoids. From these eggs larvae were obtained and were reared to the adult stage in dogs; the whole process, from the laying of the eggs to the emergence of the adult, occupied 120 to 141 days.

Dr. N. Tovar also (1924) placed captured *Dermatobia* with specimens of the mosquitos—*Psorophora posticata*, *P. lutzi*, *P. tovari*, *Aedes trivittatus*, *Stegomyia calopus*, *Culex scapularis*, and Woodland Muscoids. Bundles of eggs were laid on all the examples of *Psorophora* (fifteen in all), irrespective of sex, whilst on none of

the others (twenty specimens in all) was a single egg to be found. In fact, he stated that although the insects other than *Psorophora* were sometimes seized by the *Dermatobia*, they were treated with violence and discarded damaged, whereas the *Psorophora* were always treated gently and liberated unharmed. He also records that *Dermatobia* eggs were never found in nature save on specimens of *Psorophora* (Plate IV, fig. 1).

In view of the results of modern investigations it is interesting to record some of the names by which the natives of various parts of America referred to the fly. For instance, in Venezuela the worm was commonly known as Gusano de Zancudo, in Colombia as Gusano de Mosquito, and in Trinidad as Ver Marangouin ; all of these terms mean 'Mosquito worm.' In an old book, the 'Historia del Nuevo Mundo,' written in 1653 by a Jesuit, Father Bernabe Cobo, the following statement, doubtless based on information received from the natives, is found :—' In some of the warm lowlands there is a species of mosquito . . . somewhat reddish. In each wound produced by this mosquito, soon grows within the flesh a spine-covered worm the size of a haricot bean or even larger . . . ' (Quoted from Sambon, 1915). Knab (1913) states that in 1905 the natives of the Isthmus of Tehuantepec, Mexico, pointed out to him certain large mosquitos (*Psorophora*) as 'Madre del Gusano.' In the first reference to this fly under the binomial system of nomenclature, that of Linnaeus Junior (1781), we find :—' . . . the fly deposits on a man's skin, one after another, its eggs, or rather, its living larvae, of which it carries about 50 on its hinder portion.' (Quoted from Sambon, *loc. cit.*).

Da Matta (1920), in his account of *Dermatobia*, states that the mode of transference of the larvae may also be by direct oviposition on the skin of animals, or by indirect methods, by their deposition on leaves, from which the larvae may be picked up by passing animals, or by deposition on sweaty garments. These have been discussed by Neiva and Gomes (1917). For the first, we have been unable to find any record of a direct observation, either of eggs being found attached to the skin of animals, or of the act of deposition on the skin, although there is a record of *Dermatobia* having been seen hovering over horses with the ovipositor extended.

As to oviposition on leaves, there appear to be no records of

leaves having been found with ' packets ' of eggs adhering to them. Neiva and Gomes (*loc. cit.*) record that eggs were deposited on the sides of the vessel. They offer the explanation that, at a given moment, the female feels the necessity for oviposition irrepressible ; if then the insect which she is attempting to catch escapes, she oviposits on the nearest object. They found that eggs so laid, if kept in a moist place, produced larvae ; if, however, the conditions were dry, the eggs shrivelled and perished. They suggest that this may happen in nature, but the chances of larvae so produced being picked up by appropriate hosts do not seem to be very great. Since, however, it has been shown that the larvae can remain alive for twenty days in the egg without finding a host, this may be an alternative mode of transference.

Oviposition on ' sweaty ' clothes, too, seems to be supported by no direct observation. Neiva (1914) supports the hypothesis, saying that it would account for cases of infection of newly-born children who have never left the house. But he states that such cases are rare.

CARRIERS OF THE EGGS

The following insects have been found bearing batches of *Dermatobia* eggs in nature :—

BRAZIL :—*Psorophora posticata* (one example only, by Neiva and Gomes, 1917. Also by Peryassu, 1922).

Anthomyia heydenii (Lutz, 1917).

Anthomyia lindigii (Lutz, 1917).

Synthesiomyia brasiliiana (Lutz, 1917).

Woodland Muscoids (on numerous occasions, Neiva and Gomes, 1917).

GUATEMALA :—*Culex* sp. unknown (Morales, 1911).

PANAMA :—*Goeldia longipes* (a non-bloodsucking mosquito, Shannon, 1925).

TRINIDAD :—*Psorophora* (then *Janthinosoma*) sp. (collected by Mr. F. Urich. Knab, 1913).

VENEZUELA :—*Psorophora lutzi*, *Psorophora posticata* (Tovar, 1924).

In captivity, *Dermatobia* has laid packets or batches of eggs on the following insects :—*Musca domestica*, *Stomoxys calcitrans*, Woodland Muscoids. (Neiva and Gomes, 1917). *Psorophora posticata*, *P. lutzi*, and *P. tovari* (Tovar, 1924).

Blanchard (1896) was sent the following flies by Da Silva Araujo in 1893, as being incriminated by the natives as 'parents of the Berne' (*Dermatobia* larva):—*Lucilia ruficornis* Macq., *Sarcophaga chrysostoma* Wd., *S. plinthopyga* Wd., and an *Hystericia*.

Neiva (1910) states that in Brazil nearly all species of *Tipulidae*, *Volucella obesa*, and a species of *Mesembrinella*, are accused of producing the warbles, whilst in Matto Grosso, several species of *Echinomyia*, and in Mexico, the beetle *Atractoceros brasiliensis* were also suspected. He, however, considered these popular beliefs to be erroneous.

Finally, Dunn (1918) has suggested the possibility of a tick (probably *Amblyomma cajannense*) being a carrier. The evidence is as follows:—Dr. Clark, in the course of two trips into the interior of Panama, discovered larvae of *Dermatobia* five times in wounds in man caused by tick bites. *Psorophora* were not obtained in collections of mosquitos from the places at that time, and besides, four out of the five sites were protected by clothing so that subsequent infestation of the wound seems improbable.

GENERAL DESCRIPTION OF FIRST INSTAR LARVA (Plate IV)

The general outline is somewhat elliptical, bluntly rounded anteriorly, and gradually attenuated posteriorly, the width of the last two segments being approximately half the width of the mid-thoracic segment, as seen in profile, after maceration in caustic potash (See fig. 1).

The cephalic segment is scantily clothed with very minute spines; these appear to be more numerous dorsally and bilaterally (fig. 3A). The first thoracic segment bears a continuous band of relatively small and closely set black spines. The second and third thoracic segments are completely clothed with similar spines (figs. 3B and C). First, second and third abdominal segments show a double transverse series of large spines dorsally, and a single series ventrally; the interspaces are set with smaller spines which are much more numerous in the posterior series than in the anterior one. The fourth to sixth segments inclusive are spineless. The seventh segment is clothed with long and slender, translucent spines (fig. 3D). The terminal segment is almost covered with relatively large strongly hooked and translucent spines (fig. 3E).

The spines on the thoracic and first three abdominal segments are directed backwards, whilst those of the last two segments are directed forwards. This arrangement of the posterior groups of spines enables the larva to retain a firm hold of the inner walls of the egg-shell after partial emergence.

The main tracheal tubes of the respiratory system, which resist the action of caustic potash, show very clearly (fig. 2). On the other hand the posterior stigmata are minute and not very clearly defined; they communicate with the tracheal trunks by two long and slightly narrower felt chambers which extend to the middle of the penultimate segment.

The antennal organs (fig. 2), presumably corresponding to the antenno-maxillary organs of other Dipterous larvae as described by Keilin (1915), are placed well forward in the cephalic segment in a dorso-lateral position; the proximal portion of the organ is strengthened with an incomplete band of dark chitin, the terminal portion being translucent.

THE MOUTH PARTS (Plate V)

The mouth parts consist of the following paired appendages:—

- (1) Mouth hooks (*mh* in all figures).
- (2) 'Prestomal sclerites' (*ps* in all figures).
- (3) Stomal plates (*sp* in all figures).
- (4) Membranous bands (*mb* in all figures).
- (5) Rudimentary Hypo-pharyngeal sclerite (fig. 1C, *hs*).
- (6) Cephalo-pharyngeal sclerites (*cs* in all figures).

(1) *The Mouth Hooks.*

These are highly chitinised, blackish and strongly falciform structures, the inner edge being finely though somewhat irregularly serrated. Proximally the anterior portion is strongly produced (figs. 1A, *mh*, and 1D). There are two centrally placed foramina.

(2) *The 'Prestomal sclerites.'*

These appear to consist of very thinly chitinised, translucent plates, which may act as a sheath to the tips of the mouth hooks. They are not apparent in fig. 1A, being hidden by the stomal plates, but they are indicated in fig. 1C, *ps*.

(3) *The Stomal Plates.*

These are relatively large cone-like processes, converging distally, and with longitudinal but somewhat indefinite ridges; proximally these structures are partly surrounded by a strongly chitinated plate, which is toothed on its distal or anterior margin (figs. 1A and B, *sp*.1, and fig. 1E). Below the cones is a mass of tissue with an irregular outline, portions of which seem to bear chitinous bodies, possibly muscle attachments.

(4) *Membranous Bands.*

These very thin and very slightly chitinated structures appear to arise towards the base of the mouth hooks; they are curved, and directed outwards and slightly backwards, the tips in some cases being slightly curved inwards, and somewhat strongly chitinated.

(5) *Hypo-pharyngeal Sclerite.*

This consists of a median and very thinly chitinated plate with a pair of sub-median foramina, and lies between the anterior processes of the cephalo-pharyngeal sclerite, at the articulation with the mouth-hooks.

(6) *Cephalo-pharyngeal Sclerites.*

These consist of two plates, which are free dorsally, each consisting of three processes: a long, fairly heavily chitinated, anterior, inferior one, a short, fairly heavily chitinated, dorsal one, and a ventral one so lightly chitinated that it is difficult to see how far it extends into the thoracic region.

SOME AFFINITIES AND RELATIONSHIPS WITH OTHER FORMS

The most marked characteristics of the buccal organs of the first instar of *Dermatobia hominis*, are the presence of—

- (1) the paired and well-developed mouth hooks (*mh*);
- (2) the cone-shaped stomal plates (*sp* and *sp*1)

Another noteworthy feature is the absence of an unpaired median tooth, such as is found in most other first stage larvae, and is shown in *Hypoderma bovis* (Plate V, fig. 3, *mt*).

In his extensive paper on the larvae of Cyclorhaphous Diptera, Keilin (1915) states that in certain Acalyptrates, especially those with carnivorous larvae, one finds a precocious development of the paired mouth hooks of later stages. This condition also obtains in the first stage larva of *Calliphora*, where, however, as generally, a median tooth is present as well; *Hypoderma bovis* (Pl. V, fig. 3, *mt*, *mh*) shows both paired hooks and median tooth. No trace of the latter structure is to be seen either in *Dermatobia hominis* or in *Cordylobia anthropophaga* (Plate V, 2A-C), and this is paralleled in a figure given by Keilin of *Onesia sepulchralis* (*loc. cit.*, Pl. X, fig. 49C).

The paired mouth hooks of *Cordylobia anthropophaga* ('median buccal spine' of Blacklock, 1923, fig. 1, 3C and D) show a very remarkable modification. These structures, of which four aspects are shown in our illustration (Plate V, figs. 2A-D) are broadly dilated unilaterally at the tips and strongly toothed on the distal margin (figs. 2A-C, *mh*, and fig. 2D). Seen in profile (fig. 2B) they are strongly directed upwards, and according to Blacklock lie, when at rest, at right angles to the cephalo-pharyngeal sclerite. These processes are also very strongly developed dorsally (figs. 2B and C, *mh* 3) and bilaterally are broadly expanded (fig. 2A, *mh* 1). Further, ventrally there is a thin and broadly dilated flange (figs. 2A and C, *mh* 2) which appears to be connected with the finely spinose lower lip of the buccal cavity (fig. 2C, *bs*).

In the larva of *Hypoderma bovis*, which shows both median unpaired spines (fig. 3, *mt*) and paired mouth hooks (fig. 3, *mh*), the latter, as Carpenter and Hewitt (1914) have pointed out, are remarkable for being widely separated, directed laterally, and pointing outwards instead of upwards as in *Cordylobia anthropophaga*; or downwards and normally, as in *Dermatobia hominis*.

The peculiar form and high development of the structures we have called the stomal plates seem to be without exact parallel in other first stage larvae. They may be represented in a rudimentary form by some of the accessory pieces which Keilin has described (*loc. cit.*). For instance, there is an appendage which Keilin terms 'pièce en brosse' (*loc. cit.*, Plate VIII, fig. 37, *f*, and *f* in other figures) which may be homologous, but as we have been unable to study these forms we cannot give a definite opinion as to their homologies.

Again there are the paired structures to which we have given

the term 'membranous lobes.' These are very indefinite structures. They appear in the case of *Hypoderma bovis* (fig. 3, *mb*) to correspond in position with the parastomal sclerites described by Lowne (1890) in the third stage larva of *Calliphora erythrocephala*, whilst in *Dermatobia hominis* they appear to be in a more anterior position, lying well in front of the bases of the mouth hooks.

It is interesting to note that in these three closely allied forms, all adapted to the same mode of life, quite different dispositions of the mouth parts exist. Thus the larva of *Dermatobia* penetrates the unbroken skin of man and animals, that of *Cordylobia* the skin of rats and sometimes man, and that of *Hypoderma* the tough hide of cattle. Of the three, *Dermatobia* perhaps approximates most closely to the condition shown by *Calliphora*, differing from it markedly by the absence of the median tooth, and by the strong development of the stomal plates; *Hypoderma* agrees with *Calliphora* in the possession of lateral hooks and median tooth, but differs in the disposition of these organs, the lateral hooks being directed outwards instead of downwards. *Cordylobia* is the most aberrant of the three, the mouth hooks being modified in a most extraordinary way, and directed dorsally instead of ventrally; the latter character is no doubt correlated with the mode of penetration of the larva in a horizontal direction under the skin, as has been well described by Blacklock (*loc. cit.*).

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- * These two papers contain excellent accounts, with full bibliographies, of the early literature.

II. ANATOMY OF THE MOUTH PARTS

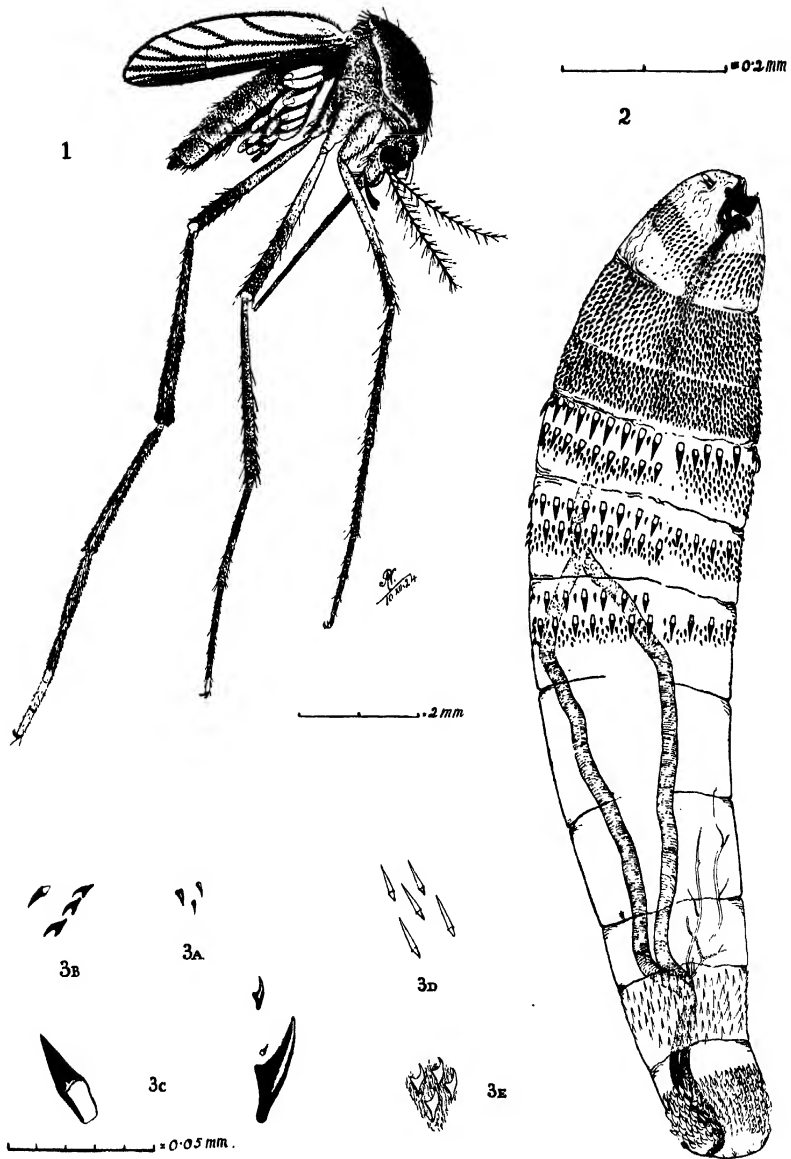
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EXPLANATION OF PLATE IV.

Dermatobia hominis.

- FIG. 1. Adult mosquito (*Psorophora posticata*) carrying a batch of eggs of *Dermatobia hominis* on the ventral surface of the abdomen. Note that in one instance the embryo larva is shown partly protruding from the egg. $\times 10$.
- FIG. 2. Larva, First Instar. Lateral view. From a specimen macerated in caustic potash. Showing the arrangement of the dermal spines and the main sub-lying tracheal tubes. $\times 140$.
- FIG. 3A. Dermal spines from the cephalic segment.
- FIG. 3B. Dermal spines from a thoracic segment.
- FIG. 3C. Dermal spines from a thoracic segment.
- FIG. 3D. Dermal spines from the penultimate abdominal segment.
- FIG. 3E. Dermal spines from the terminal abdominal segment.

Figs. 3A-3E (inclusive) $\times 500$.



EXPLANATION OF PLATE V.

Mouth Parts of First Instar Larva of Dermatobia hominis.

FIG. 1A. Profile.

FIG. 1B. Ventral.

FIG. 1C. Dorsal.

FIG. 1D. Mouth hook.

FIG. 1E. Two views of the proximal portions of the stomal plates, detached from the cone-shaped process.

Mouth Parts of First Instar Larva of Cordylobia anthropophaga.

FIG. 2A. Ventral.

FIG. 2B. Dorsal.

FIG. 2C. Profile.

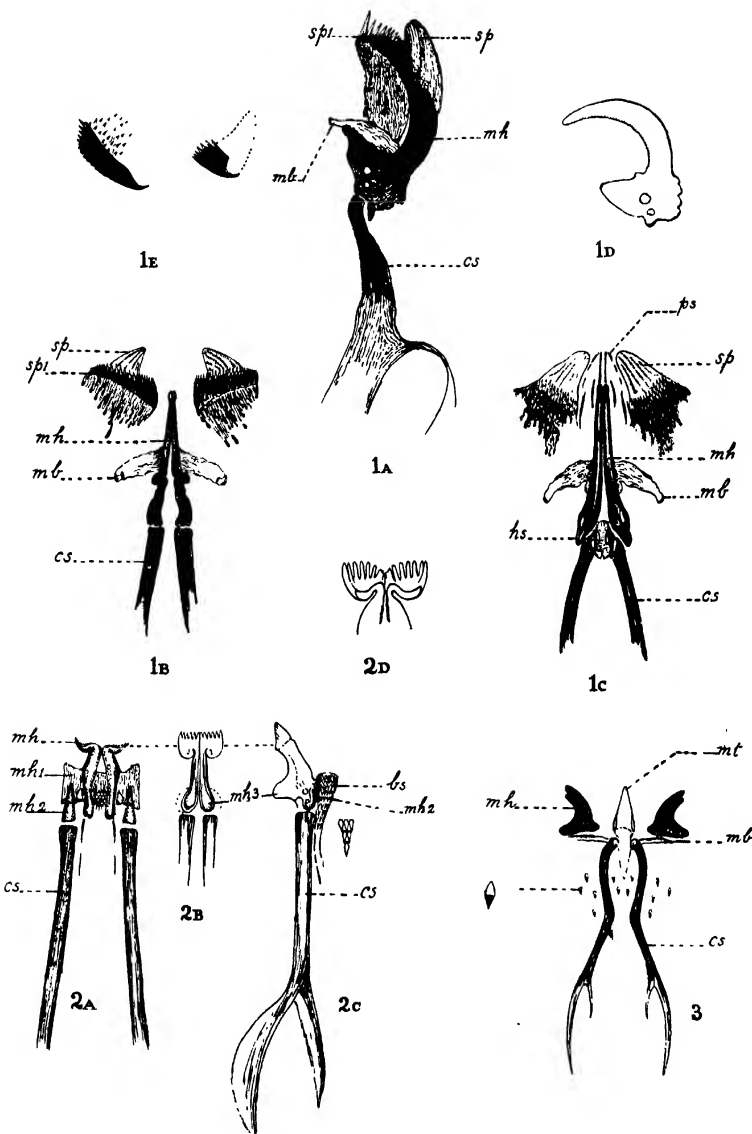
FIG. 2D. Terminal portion of mouth hooks, ventral view, showing the arrangement of the teeth $\times 1000$.*Mouth Parts of Third Instar of Hypoderma bovis.*

FIG. 3. Dorsal.

All the figures with the exception of Fig. 2D. $\times 500$.

EXPLANATION OF LETTERING.

- cs* = cephalo-pharyngeal sclerites.
- bs* = buccal spines.
- bs* = rudimentary hypo-pharyngeal sclerite.
- mb* = paired membranous bands.
- mb* = mouth hooks.
- mb1* = lateral proximal extension of the mouth hooks.
- mb2* = ventral proximal flange of the mouth hooks.
- mb3* = dorsal proximal process of the mouth hooks.
- mt* = median tooth.
- ps* = prestomal sclerites.
- sp* = stomal plates.
- spl* = proximal pieces of stomal plates.



MISCELLANEA

PLACOBDELLA PARASITICA

Dr. Aitken Clark has presented to the Liverpool School of Tropical Medicine a large leech measuring, when fully expanded, about seven inches, which was found sucking human blood, in Para, Brazil.

The specimen proved to be *Placobdella parasitica* (Say) 1824. It is most commonly found attached to species of *Chelydra*, etc.

T. SOUTHWELL.

A CASE OF EMPYEMA SIMULATING ABSCESS OF THE LIVER

The patient, a male aged thirty, Brazilian, who had resided in Amazonas for the past ten years, was admitted to hospital 29.12.21.

History.—Patient dates his present illness from an attack of dysentery six months ago. Symptoms began with pain in the right hypochondriac region, debility, fever every afternoon, profuse sweating, loss of weight and swelling of the abdomen.

On admission.—Patient very much emaciated; abdomen generally distended, but markedly so in the right hypochondriac and epigastric regions. The right hypochondrium is occupied by a swelling, resistant, somewhat resilient, dull on percussion, reaching almost to the level of the right anterior spine of the ilium and across the mid-line, the swelling bulges in the right flank. Dullness on percussion is complete as high as the right nipple in front, and almost to the inferior angle of the scapula posteriorly. No fluctuation is perceptible over the swelling, which moves slightly with respiration.

No cough; no sputum; dullness and faint breath sounds over the base of the right lung as high as the inferior angle of the scapula.

Treatment and Progress.—On 31.12.21 an exploratory puncture was made one inch below the right costal margin in the anterior axillary lines, in the most prominent and most tender part of the tumour. One-and-a-half litres of reddish brown pus were aspirated with great relief of symptoms. Although no amoebae were present, on the assumption that the condition was probably liver abscess, a

grain of Emetine was administered hypodermically, and repeated daily till 21.1.22. The evening temperature was normal for a week, but then rose again. Another litre of pus was aspirated, but again two days later the temperature rose. It was then considered advisable to operate. On 21.1.22 a needle was inserted one inch below the costal margin in the anterior axillary line and a small amount of pus aspirated with a syringe. Under chloroform anaesthesia an incision was made, four inches long, parallel to the costal margin, its centre being just below the insertion of the needle. When the incision was deepened it was seen that the needle penetrated the diaphragm, which bulged down below the level of the incision. The diaphragm was incised and two litres of pus were evacuated. A finger passed through the incision and upwards encountered a large empty space, with the lung collapsed towards the apex. Inferiorly the diaphragm, partly adherent to the upper surface of the liver, was found to bulge downwards, below the level of the skin incision, to a distance of about two inches. A drainage tube was inserted and the remainder of the incision closed. For a week a fair amount of pus was discharged, the patient being encouraged to do breathing exercises to expand the lung.

2.2.22. Discharge much diminished ; drain and stitches removed.

5.3.22. Patient left hospital ; practically no discharge ; feeling very well and weighing 20 kilos more than on admission.

15.3.22. Patient returned to report. In the interval he had been riding on horseback every day. Wound closed and patient feeling very fit.

R. M. BURNIE.

THE GOLUBACSER FLY

. . . There is, in Servia and the Banat, a minute fly,* from whose destructive assaults on the cattle the inhabitants have suffered immense losses. A traveller, arriving at Golubacs, on the Danube, thus speaks of it :—

“ Near this place we found a range of caverns, famous for producing the poisonous fly, too well known in Servia and Hungary

* *Simulium columbascense* Köll.

under the name of the Golubacser fly. These singular and venomous insects, somewhat resembling mosquitos, generally make their appearance during the first great heat of the summer, in such numbers as to appear like vast volumes of smoke. Their attacks are always directed against every description of quâdruped, and so potent is the poison they communicate, that even an ox is unable to withstand its influence, for he always expires in less than two hours. This results, not so much from the virulence of the poison, as that every vulnerable part is simultaneously covered with these most destructive insects ; when the wretched animals, frenzied with pain, rush wild through the fields till death puts a period to their sufferings, or they accelerate dissolution by plunging headlong into the rivers " '*

'The Romance of Natural History,' by P. H. Gosse, F.R.S., 2nd ed., 1861, p. 111. Compare these ANNALS, XVIII, No. 3, 1924, p. 323.

J. W. W. STEPHENS.

* Spence's *Travels in Circassia*, i, p. 59.

ON
PROTEOCEPHALUS MARENZELLERI,
P. NAIÆ AND *P. VIPERIS*

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***PROTEOCEPHALUS MARENZELLERI* (Barrois, 1898)**

This species, one of the largest known Proteocephalids, was first proposed (as *Ichthyotaenia marenzelleri*) by Barrois (1898) who supplied a very brief account of its structure from material collected by Calmette in 1897 from *Ancistrodon piscivorous* Holbr., the 'Water Viper,' a snake found in the southern United States. Ten years later, Schwarz (1908) published a more complete description, with three figures, solely based, however, upon the identical material studied by Barrois. Five years later still, Beddard (1913b) supplied some further details of structure from the examination of a number of immature specimens (the longest measuring about 250 mm.) found in a water viper which had died in the London Zoological Gardens. Since Beddard's specimens were immature and La Rue (1914) expressly recommends a further study of new material, the following description of the anatomy of one large fully-mature example, which I have found recently in the Wellcome Bureau collection of Helminths and which was collected from a water viper which had also died in the London Zoological Gardens, is worth publishing.

My single specimen measured between 300 mm. and 400 mm. in length and was well preserved in spirit. It shows well a striking feature of this species, viz., the very small proportion of the strobila which consists of mature and ripe proglottides. As Beddard remarks concerning his specimens, 'in proglottides situated 8 inches or so

from the scolex [the largest worm being 10 inches in length in spirit] there were merely traces of the reproductive organs,' and in my own single specimen, though it must have measured about 350 mm. (i.e., over 14 inches, in spirit) in total length, yet I did not obtain from the strobila more than about a dozen ripe proglottides and as many which could be described as mature, the rest of the strobila consisting of immature proglottides.

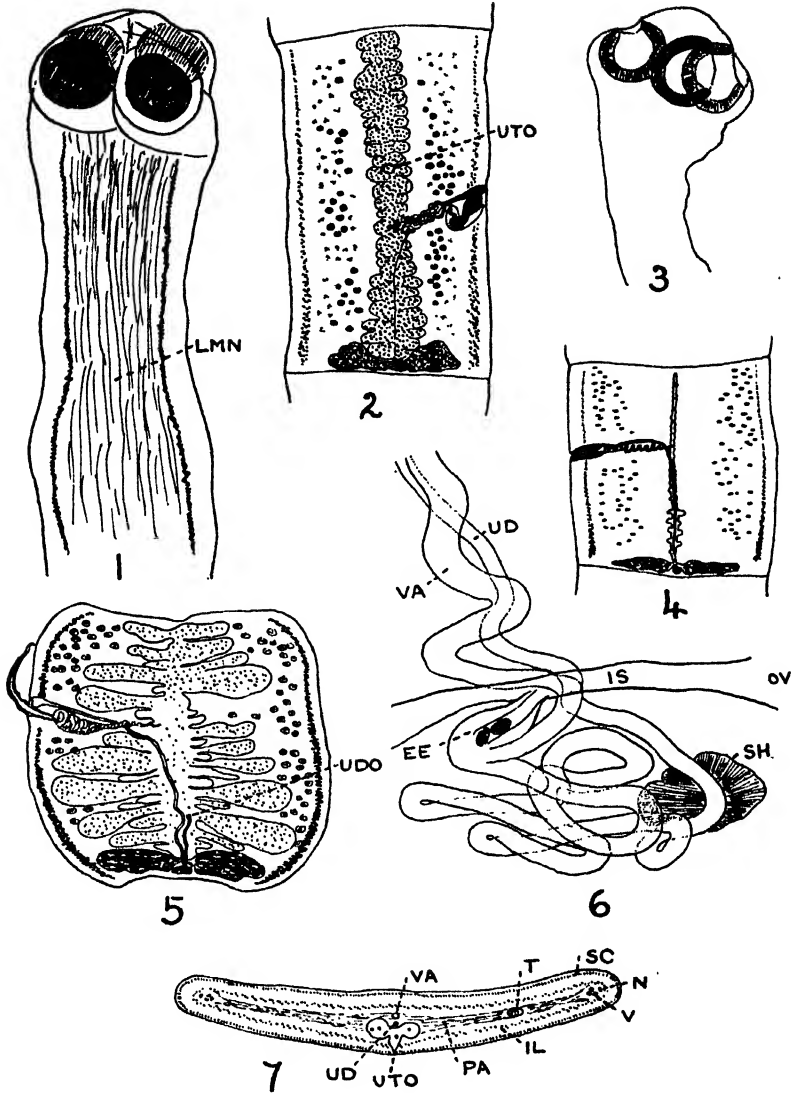
The scolex (fig. 1) was present and measured about 1.9 mm. in breadth. The four large suckers are borne on protrusible lobes on the anterior end of the scolex and face upwards and outwards, the apex of the scolex being quite insignificant, i.e., no 'rostellum' is present. I did not sectionize the scolex to ascertain if a minute apical organ were present. The suckers measure about 0.913 mm. in breadth. Spinelets are entirely absent. The unsegmented neck is about 7 mm. long, with an average breadth of about 1.4 mm.

The strobila is, in transverse section, extremely flat and has a maximum breadth of a little over 3 mm. (3.068 mm.). The immature proglottides which, as already stated, compose by far the greater part of the strobila, vary in size and shape from $\frac{1.9 \text{ mm. broad}}{0.767 \text{ mm. long}}$ in front to $\frac{3.068 \text{ mm. broad}}{1.180 \text{ mm. long}}$ behind, and thus are all broader than long. Mature proglottides are more square in shape ($\frac{2.95 \text{ mm. broad}}{1.71 \text{ mm. long}}$ and $\frac{2.8 \text{ mm. broad}}{2.0 \text{ mm. long}}$), while ripe proglottides are, more anteriorly, distinctly broader than long ($\frac{3.068 \text{ mm. broad}}{1.8 \text{ mm. long}}$) and, more posteriorly, longer than broad ($\frac{2.124 \text{ mm. broad}}{4.0 \text{ mm. long}}$). The genital apertures are, as usual, irregularly alternate and open midway in the lengths of the proglottides, and the cirrus and vaginal apertures irregularly alternate as to which is anterior. The cirrus sac in mature and ripe segments is extremely broad antero-posteriorly, measuring 0.448 to 0.680 mm. in length and 0.149 to 0.298 mm. in maximum breadth. In some proglottides the sac stretches over a quarter of the breadth of the proglottis but usually a somewhat less distance. The contained cirrus is, next the opening, bulbous in form, and continuous with a straight portion which is connected with several coils of the ductus at the base of the sac. The cirrus was not everted in any of my preparations. The vas deferens

outside the sac is not very voluminous and stretches to the middle line of the proglottis. The vagina is dilated only next its opening. Occasionally a small genital atrium or depression seems to be present, but usually the two apertures appear to lie next the surface at the same horizontal level. The young ovary is rather flattened antero-posteriorly but becomes less so in ripe proglottides, and is of the lateral extent shown in fig. 2. The isthmus joining the two lobes of the ovary is thin and canalicular when seen in toto-preparations and the entire ovary is seen to be very flat in transverse sections. Both the uterine canal and the vagina lie dorsal to the ovarian isthmus (the vagina being ventral to the canal in front of the ovary) and a shell-gland is present. The testes are numerous and lie in two lateral fields and measure on an average about 44 by 22 microns. The vitelline strands are thicker posteriorly than anteriorly.

The uterus in mature segments is of the usual type, viz., a narrow hollow stem lying in the median longitudinal line of the proglottis, continuous with the uterine canal, and with no openings to the exterior. In ripe proglottides, on the other hand (fig. 2), the hollow median stem has become dilated into a broad trunk of considerable size (occupying a fifth or sixth of the breadth of the proglottis and most of the space between the dorsal and ventral surfaces), the wall of which bears irregular lateral very short lobose protuberances, the whole cavity being filled with eggs. Serial transverse sections also reveal the fact that at this stage the uterus opens to the exterior by a single very large and conspicuous ventral pore, situated anterior to the cirrus sac level and nearly midway between this and the anterior limit of the proglottis. I am not certain as to whether other additional pores are subsequently formed (and the ventral uterine wall approaches very close to the ventral subcuticula in two or three places, though no openings were present in my sections) but I doubt it, both because the proglottides appear to be fully ripe and because of the large size of the very well-defined single existing pore. The uterine eggs measure about 22 microns in external diameter, the embryos about 11 microns.

In transverse section the mature and ripe proglottides are seen to be extremely flat. Beddard remarks that in his immature specimens he could find 'no marked layer of longitudinal fibres in the body generally' and quotes Schwarz as saying that 'die innere

FIGS. 1, 2. *Proteocephalus marenzelleri*." 3 to 7. *Proteocephalus naiaes*.Fig. 1 ($\times 12$). Scolex. Compare the magnifications of this and fig. 2, and figs. 3, 4 and 5.Fig. 2 ($\times 12$). Ripe proglottis. Dorsal view. The testes are degenerating. Note the small size of the uterine diverticula.Fig. 3 ($\times 87.5$). Scolex in outline.Fig. 4 ($\times 12$). Mature proglottis, unflattened. Dorsal view.Fig. 5 ($\times 12$). Ripe proglottis, much flattened. Dorsal view.Fig. 6 ($\times 180$). Ducts in the region of the ovarian isthmus. Ventral view.Fig. 7 ($\times 27.5$). Transverse section through a young ripe proglottis immediately in front of the ovary.

EE.—egg-ejector ("schluckapparat"); IL.—internal layer (sheath) of longitudinal muscles; IS.—isthmus of ovary; LMN.—longitudinal muscles of neck; N.—nerve; OV.—ovary; PA.—modified parenchymal core in medulla; SC.—nuclear layer of subcuticula; SH.—shell gland; T.—testes; UD.—uterine canal; UDO.—opening of uterine canal into median chamber of uterus sac; UTO.—opening of uterus to exterior; V.—vitellaria; Va.—vagina.

Längsmuskulatur ist schwach.' In all my sections through mature and ripe proglottides there is a very distinct layer of internal longitudinal muscles—much more distinct than that in Beddard's '*Solenotaenia*' *viperis* (*vide infra*), because definite bundles of two, three or more fibres are present and are relatively numerous. The medulla contains a core of specialized parenchyma in which the meshes are transversely elongated. I only observed the specialized longitudinal musculature of the neck region in a toto-preparation.

***PROTEOCEPHALUS NAIÆ* (Beddard, 1913)**

Syn., *Ophidotaenia naiae* Beddard, 1913.

Of this species I possess more than two dozen specimens, all taken from the anterior and middle intestines of ten (fourteen examined) full-sized cobras (*Naia tripudians*), supplied by snake-charmers of the United Provinces, India. This species has already been described by Beddard (1913a) from three specimens obtained from a cobra which died in the Zoological Gardens, London, but a re-description is necessary owing to the original account being deficient in some respects. Most of my specimens were found in the intestine just behind the stomach and isolated detached proglottides were also found in the faeces on several occasions. My largest (unflattened) specimen measured 180 mm. in total length, with a maximum breadth of 2.5 mm., but other specimens (mostly without ripe proglottides) measured considerably less and one immature specimen only measured 43 mm. Beddard's largest specimen measured 110 mm., with a maximum breadth of 1.5 mm.

In the following account of the species I shall for the most part only deal with those features which require a more complete description than Beddard has given, or which, in my opinion, have been misunderstood by him. As Beddard remarks, the 'rostellum' (by which term I mean simply the terminal part of the scolex, anterior to the suckers) is never very conspicuous (fig. 3) and when retracted consists solely of a very restricted non-projecting area lying between the suckers. In my specimens (toto- and in sections), as in Beddard's, an apical body is absent, though a number of gland-like cells appear to be clustered in the position normally occupied by

the apical organ. Cuticular spinelets were absent. In seven of my balsam preparations, the scolex measured 0.248 to 0.303 mm. in breadth and 0.153 to 0.201 mm. in length (from apex to lower edge of suckers), and the maximum diameter of the suckers varied between 0.106 mm. and 0.153 mm. The suckers are borne on lobes of the scolex base (each sucker, however, occupying the bulk of the lobe) and are undoubtedly protrusible. The unsegmented neck in my specimens is of considerable length, varying between 3.5 mm. (in one case) and 6 mm. (in most cases) according to the state of contraction, and 0.116 to 0.614 mm. in breadth. According to Beddard the neck is 'short.'

The mature and ripe proglottides are of considerable size and are only found in the extreme hind regions of most worms, the greater part of the strobila being composed of large and yet immature proglottides. Only in one of my two dozen specimens were the proglottides in a ripe condition. The immature proglottides (unflattened) in most worms varied between $\frac{2.242 \text{ mm. broad}}{0.295 \text{ mm. long}}$ and $\frac{1.770 \text{ mm. broad}}{1.121 \text{ mm. long}}$, and more or less mature proglottides between $\frac{2.655 \text{ mm. broad}}{0.413 \text{ mm. long}}$ (considerable contraction) and $\frac{0.531 \text{ mm. broad}}{3.009 \text{ mm. long}}$ (considerable extension), but the average shape of the mature and ripe proglottides is square or a little longer than broad (figs. 4, 5). The genital openings are irregularly alternate and open, often on a distinct projection, either midway in the length of the proglottis or a little anterior to this point. The cirrus sac and vagina irregularly alternate as to which is anterior. The cirrus sac, in unflattened and not unduly contracted or extended proglottides, extends across about a quarter of the breadth of the proglottis and, when fully developed, measures about 0.498 to 0.531 mm. long and 0.083 to 0.107 mm. broad. In flattened (between glass slides) and very contracted or extended proglottides the sac varies enormously in size, from being almost globular in form and therefore very short, to very elongated in form and extending across at least one-third the width of the proglottis. In the cirrus sac both the cirrus (unarmed) and the ductus are usually coiled. In many of my flattened proglottides the cirrus is everted to its full extent and in some cases is longer than one-third the width of the proglottis,

and then the sac is practically invisible, from which I conclude (though the eversion may have been due to the artificial flattening) that, in these cases at least, the cirrus sac itself has been everted, though Beddard says that he has seen no evidence of this. The wall of the cirrus sac is thin but muscular. A small cloaca genitalis is present. The coils of the vas deferens in unflattened preparations are not very voluminous and are just visible as far as the middle line of the proglottis. In elongated proglottides the vas deferens coils form a bunch in the middle line. The vagina opens at the same horizontal level as the cirrus, but away from the opening it lies ventral to the cirrus sac and to the vas deferens coils. The vagina is very slightly dilated near its opening but in no other region and, except in very elongated proglottides, is sinuous or slightly convoluted just anterior to the ovary. The testes are about 120 in number and in unflattened preparations measure, on the average, about 62 by 36 microns. They are situated in two quite separate lateral fields. The vitellaria (cir. 14 by 11 microns) are, as usual, arranged in two thin lateral strands, which, however, are distinctly broader posteriorly than anteriorly. The ovary consists, as usual, of two lobes connected medianly by an isthmus. In surface view the lobes are narrow antero-posteriorly and extend laterally, in mature proglottides, only a little more than half-way to the proglottis edge. In sections they are seen to be very thin dorso-ventrally and to lie nearer the dorsal than the ventral surface of the strobila, though the isthmus connecting the lobes bends ventrally to allow of the dorsal passage of the uterine canal and vagina. The lobes are distinctly follicular. Beddard says that he 'could not find any signs of a shell-gland' and he endeavours to correlate its supposed absence with the presence of a 'glandular investment' on the walls of the uterine diverticula, which he assumes to take the place of a shell-gland. For my part, I have had no difficulty in finding a very distinct shell-gland (diagrammatically represented in fig. 6) in most of my preparations, and, on the other hand, I have been unable to find any investment of the uterine walls with cells which can be described as glandular. A distinct egg-ejector ('schluckapparat') is also present. The uterus in my ripe proglottides (fig. 5) consists of (a) a wide median uterine sac extending the whole length of the proglottis from the ovary anteriorly, which carries on either side from 16 to 25 lobose diverticula

of very different sizes (I could not detect any diverticula ventral to the ovary), and (b) a uterine canal, which, with the vagina, passes *dorsal* to the ovarian isthmus, and opens into the median uterine sac some distance in front of the ovary. Nearly all the eggs are collected in the diverticula, in which they are freely scattered (not in clusters), and they possess two distinct shells, the outermost thick shell measuring about 25.6 microns in diameter, and the contained embryo 9 to 11 microns. The median sac of the uterus has a number of pointed downgrowths (fig. 7) which open on what is usually considered to be the ventral surface of the strobila. The position of the uterine pores is indeed the sole certain criterion of determining the orientation of the strobila.

In transverse sections (fig. 7) through mature proglottides the usual two layers of longitudinal muscles are to be seen—a very thin layer just external to the nucleated region of the subcuticula and internal to an equally thin circular muscle layer, and a thicker, though somewhat attenuated, internal layer of longitudinal muscles, demarcating the cortex from the medulla. The parenchyma is in most regions of a uniform wide-meshed character, but in the centre of the medulla there is a kind of core of closer-meshed parenchyma, with the meshes transversely elongated. This core of differentiated parenchyma is apparently identical with that figured by Beddard (1913b, p. 167, text-fig. 38) for '*Ichthyotaenia* sp.' (i.e., *P. marenzelleri*) only Beddard assumes (in the absence in his immature specimens of an internal longitudinal muscle sheath) that it represents the whole of the medulla, whereas in *P. naiae* and in my mature specimens of *P. marenzelleri* (*vide supra*) it is obviously only the internal region of it. I am ignorant of the significance of this altered parenchymal core.

***PROTEOCEPHALUS VIPERIS* (Beddard, 1913)**

Syn., *Solenotaenia viperis* Beddard, 1913.

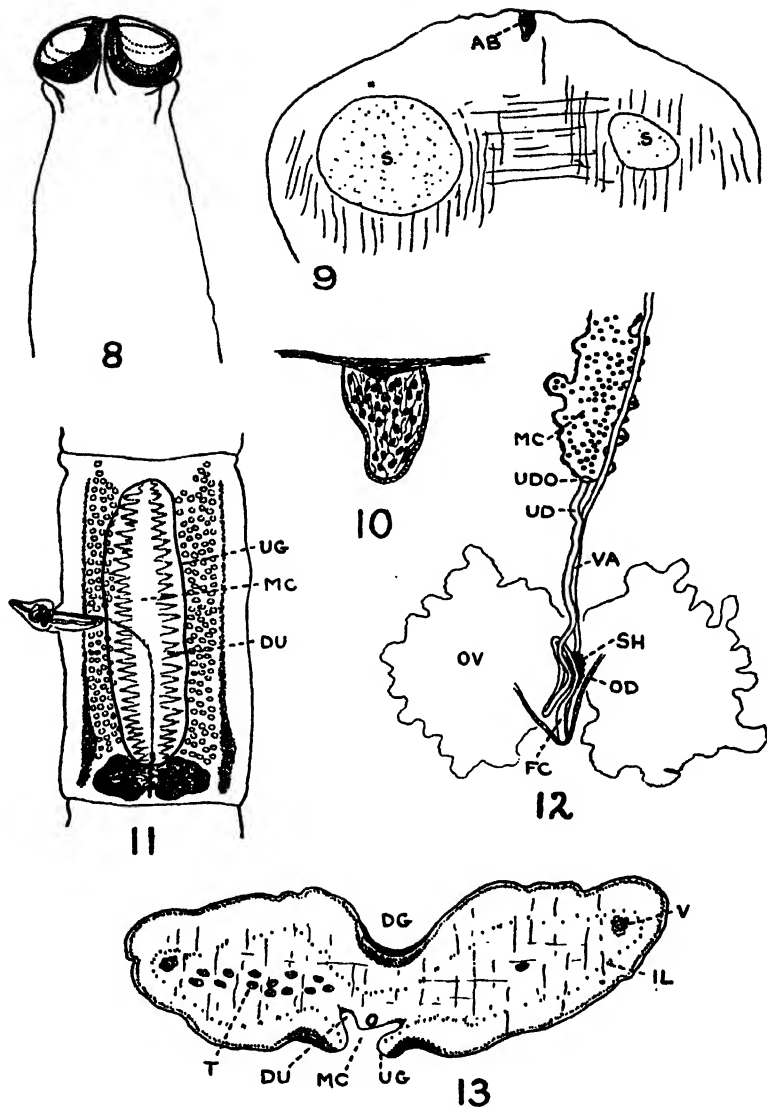
Beddard (1913c) has provided a full description of this remarkable species, and the following account only professes to supply details which Beddard has omitted and to confirm and, if possible, emphasize, his statement of the peculiar character upon which he founded his new genus *Solenotaenia*. I possess a large number of specimens

of this species contained in the helminthological collection made by Dr. L. W. Sambon almost entirely from animals which had died in the London Zoological Gardens, and now in the Wellcome Bureau of Scientific Research.

Proteocephalus viperis (as I propose to call this species) is a parasite of the Crossed Viper, *Lachesis alternatus*, from Central or South America. One nearly-entire worm in my collection measured 170 mm. in total length (in spirit) and others must have reached 200 mm. and possibly more, so that my specimens were somewhat longer than those studied by Beddard. The maximum breadth of my specimens was 2.41 mm., and was always found in the region of immature proglottides. Mature and ripe proglottides only occur in about the last quarter of the worm's length. There are no external signs of segmentation, save the small lateral notches and the uterine grooves which demarcate ripe proglottides.

The scolex (fig. 8) consists almost entirely of the four large hemispherical suckers, which occupy the greater part of the four lobes which bear them. The terminal area between the suckers is extremely small and does not protrude. The scolex measures 0.860 to 1.527 mm. in breadth and 0.531 to 0.713 mm. in length (from tops to bases of suckers). The suckers measure 0.415 to 0.664 mm. in breadth and look upwards and outwards. Spinelets are entirely absent. As Beddard remarks, a minute funnel-shaped apical organ is present, which is so small that it is almost invisible in toto-preparations. Its appearance, in longitudinal section, is shown in figs. 9 and 10. An unsegmented neck is present, varying in different specimens from 1.7 mm. to 4.7 mm. in length and 0.767 to 1.534 mm. in breadth, but the average length is about 3 mm.

As already remarked, the broadest part of the strobila is in the region of immature proglottides. The proglottides are here indicated by the presence of faint transverse segmentation lines and more posteriorly by genital rudiments and are all much broader than long, measuring from $\frac{2.419 \text{ mm. broad}}{0.236 \text{ mm. long}}$ and $\frac{2.124 \text{ mm. broad}}{0.088 \text{ mm. long}}$ to $\frac{1.534 \text{ mm. broad}}{0.354 \text{ mm. long}}$ (all measurements of unflattened material). Mature and ripe proglottides are not nearly so broad, the former being either



FIGS. 8 TO 13. *Proteocephalus viperis*.

Fig. 8 ($\times 17.5$). Scolex in outline.

Fig. 9 ($\times 56$). Longitudinal section through the scolex showing the minute apical organ.

Fig. 10 ($\times 260$). The apical organ in longitudinal section.

Fig. 11 ($\times 27.5$). Ripe proglottis with the uterus entirely split along its whole length and empty of eggs. Note the small diverticula.

Fig. 12 ($\times 56$). Ducts in the region of the ovary, from the dorsal view. In this proglottis the uterus has not yet split to the exterior.

Fig. 13 ($\times 39$). Transverse section through a ripe proglottis behind the cirrus sac. Note the open uterus, the small uterine diverticula and the weak internal longitudinal muscle sheath.

AB.—apical organ; DG.—dorsal groove (artefact ?); DU.—diverticula of uterus; FC.—fertilization chamber; IL.—internal layer (sheath) of longitudinal muscles; MC.—median chamber of uterus; OD.—oviduct (?); OV.—ovary; S.—sucker; SH.—shell-gland; T.—testes; UD.—uterine canal; UDO.—opening of uterine canal into median chamber of uterus sac; UG.—uterine groove, edge of; V.—vitellaria; VA.—vagina.

approximately square in shape or longer than broad, and measuring from $\frac{1.121 \text{ mm. broad}}{0.885 \text{ mm. long}}$ to $\frac{1.534 \text{ mm. broad}}{1.888 \text{ mm. long}}$ and $\frac{1.230 \text{ mm. broad}}{2.006 \text{ mm. long}}$, and the latter (fig. 11) always being longer than broad and measuring from $\frac{0.885 \text{ mm. broad}}{1.652 \text{ mm. long}}$ to $\frac{1.357 \text{ mm. broad}}{4.838 \text{ mm. long}}$. In my material the genital apertures are situated almost on the middle transverse line of the proglottis but not quite, being a little in front, and the cirrus aperture is usually in front of the vaginal, though the reverse condition does occur. The cirrus sac is very broad antero-posteriorly and measures in my preparations 0.298 to 0.365 mm. in length and 0.149 to 0.182 mm. in breadth, and it extends across from one quarter to one-third of the breadth of the proglottis according to the state of contraction of the latter. The cirrus sac wall is very thin, though quite well-defined, and apparently contains no muscle-fibres. The sac contains three parts of the cirrus apparatus: (a) an external thick-walled convoluted part which forms the outer walls of the extruded cirrus, (b) a long thick-walled (less thick than the first part) straight tube (the cirrus canal), and (c) coils of the ductus. The first two parts have attached to them ejector muscle-fibres (Beddard's 'layer of glandular cells'?). The cirrus when everted (which may equal in length half the breadth of the proglottis) is slender distally but dilated at the base which contains the ductus coils (fig. 11), and the sac, contained inside the proglottis in this condition, is relatively narrow (only 0.041 mm. broad and 0.298 mm. long in one specimen which reached a quarter of the distance across the proglottis). The sac itself then is not everted. The cirrus is not armed. The coils of the vas deferens are not very voluminous and extend to about the middle of the proglottis. The vagina opens on the same horizontal level as the cirrus and shows no marked dilatation anywhere in its course, though in contracted proglottides (not in extended) it becomes convoluted anterior to the ovary. It occasionally opens on a papilla and there is no genital atrium. The ovary in mature non-elongated proglottides is only a little more than half the breadth of the proglottis and is of the shape shown in fig. 11. There is no narrow canalicular isthmus, the follicles of the ovary extending across the middle line over a broad area. The vitellarian strands (thickened posteriorly)

have been sufficiently described by Beddard, and are of course medullary in position. The testes, as Beddard states, are very numerous, lie in two distinct fields, and measure in toto-preparations about 44 by 25 microns. I must also mention that both the vagina and the uterine canal (*vide infra*) lie on the dorsal side of the ovary (the former lying for the most part ventral to the latter), that in two of my preparations the oviducts* apparently join the vagina at the level of the hind end of the ovary (an unusual position) and that there is a recognizable shell-gland. In fig. 12 I have depicted these ducts as well as I am able to make them out, but I cannot guarantee the exact positions of the coils, nor could I detect the vitelline ducts.

Beddard has fully described the uterus and its extraordinary later development in this species, and I intend only to make one or two corrections in his account and to emphasize the features in which this uterus differs from that of other known Proteocephalidae. As Beddard says, the early development of the uterus as a median hollow stem is like that of other Proteocephalids, but he omits to lay stress upon the fact that whereas the uterus of most other Proteocephalids remains devoid of eggs until the diverticula are well developed, the uterus of '*Solenotaenia*' (like that of *P. marenzelleri* and some other snake Proteocephalids) becomes crammed with eggs while the diverticula are either entirely absent or only represented by minute irregularities of the wall (fig. 12). This fact in itself indicates that the '*Solenotaenia*' uterus is distinct from that of the majority of Proteocephalids. The next stage of development of the '*Solenotaenia*' uterus is, not the development of large diverticula, but the splitting and opening to the exterior of its entire ventral wall (the process commencing anteriorly and proceeding posteriorly, until the entire length is exposed), so that the whole cavity of the stem uterus becomes continuous with the outer world and in consequence devoid of the eggs which are at once liberated (figs. 11, 13). There is thus formed, as the final stage of development of the uterus, a deep and broad uterine groove, with smooth thickened edges, situated on the ventral side of the proglottis along nearly its entire length, i.e., from the posterior opening of the uterine duct to near

* These ducts (which are very distinct in one preparation) may possibly be the vitelline ducts, though they appear to come from the ovary and I cannot trace any connection with the vitellaria. I also admit that I cannot see these ducts in most of my preparations, nor in serial transverse sections.

the anterior end of the proglottis. This conspicuous uterine groove, as Beddard points out, is quite distinct morphologically from the apparently similar longitudinal grooves in certain *Bothriocephalids* and in many *Proteocephalidae*, since in these latter it is only a continuous depression of the body-wall which harbours the uterine pores, whereas in the former it is equivalent to the fused uterine pores themselves and represents the actual cavity of the uterus. Correlated with this formation of the uterine groove*

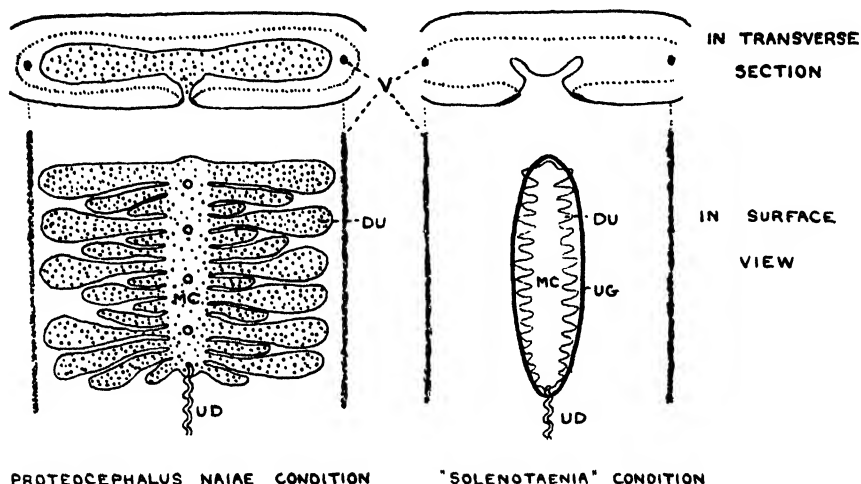


DIAGRAM to contrast the conditions of the ripe uteri of a normal *Proteocephalid* and of "*Solenotaenia*."

DU.—diverticula of uterus; MC.—median chamber of uterus; UD.—uterine canal; UG.—uterine groove, edge of; V.—vitellaria.

in '*Solenotaenia*' and the early formation of the large uterine pores in allied species is the stunted development of the diverticula which are so conspicuous a feature in the fully-formed uteri of most other *Proteocephalids*. This difference of development of the uterus in this species, compared with the developments of the uteri of most other *Proteocephalids*, would afford a very much better basis for the founding of a distinct genus (cf. Lühe's characterization of the genera of the *Ptychobothriinae* e.g.) than the trivial scolex characters,

* In my figure 13 of a transverse section it will be observed that there is, in my material, a very distinct dorsal groove bordered by a thickened area of the subcuticula. This dorsal groove is possibly the result of local contraction, since Beddard's figures do not indicate its presence in his material.

and even the testes distribution, which have been utilized up to the present, and I would readily adopt Beddard's new genus *Solenotaenia* were it not for the facts that: (1) the stunted uterine diverticula found in '*Solenotaenia*' are also to be found in several other Ophidian Proteocephalids which do not possess the uterine groove (e.g., in *P. marenzelleri*, *P. calmettei* and '*Crepidobothrium*' *gerrardii*), and that (2) there appears to be every transition from these stunted diverticula (cf. e.g., *P. racemosa*, *P. nattereri* and the '*O. monnigi*' recently described by Fuhrmann, 1924) up to fully-developed diverticula (as in the '*O. punica*' recently re-described by Southwell and Adler, 1923, and many other species of '*Ophiotaenia*'), and that (3) the uterine groove represents physiologically, if not morphologically, after all only a fusion of uterine pores and is therefore only an individual, i.e., specific, peculiarity of an external character.

Beddard says nothing about the development of the uterus into two definitive portions—the dorsal *uterine canal** (representing the posterior portion of the primitive stem) and the ventral *uterine sac*—a development common to many other Proteocephalids, though not to all. He, however, states that in this species and in '*Ophiotaenia*' *naiae* (= *Proteocephalus naiae*, *vide supra*) the minute and large uterine diverticula respectively are invested with cells which seem to be 'exactly like those of the shell-gland in other tape-worms,' and he suggests that they may have a similar function, i.e., that the eggs may, in these two species, acquire their shells in the uterus instead of in the usual place. I have observed these cells investing the uterine wall in *P. viperis*, but I am not convinced of their glandular nature, and, as Beddard remarks, a shell-gland is apparently present* in '*Solenotaenia*,' and is certainly present in *Proteocephalus naiae*, though Beddard failed to observe it in this latter case.

The fully-formed eggs of *Proteocephalis viperis* are fairly thick-shelled and measure about 21.7 microns in diameter, and the contained hooked embryos about 12.8 microns. In transverse section (fig. 13) the general parenchyma of the proglottis is seen to be wide-meshed and to be divided into the usual two regions of cortex and medulla by the presence of a very weakly-developed internal

* Beddard apparently figures this canal in transverse section in his text-figure 50 (p. 252) but is under the impression that it represents a convolution of the vagina. In my serial transverse sections I have followed both ducts through their entire course and have seen the uterine canal opening into the uterus sac.

layer of longitudinal muscles, a layer consisting of widely-separated small bundles, each containing only two or three fibres, and occasionally of single fibres.

The foregoing three species are provisionally placed in the genus *Proteocephalus* (syn. *Ichthyotaenia*) and not in '*Ophiotaenia*' (La Rue, 1911) or '*Crepidobothrium*' (vide Nybelin, 1917) for the reasons stated by me in a paper published elsewhere (Woodland, 1925).

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HUMAN TRYPANOSOMIASIS IN THE LUANGWA VALLEY, NORTHERN RHODESIA

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I. PHYSICAL ASPECTS OF THE VALLEY

The Luangwa river rises in the hilly country near the junction of the Rhodesia-Nyasaland-Tanganyika Territory boundary at an altitude of from 5,500 to 5,900 feet and flows in a general south-westerly direction to its confluence with the Zambesi at Feira, 1,500 feet above sea level. On either side its valley is bounded by ranges of hills which run roughly parallel to the course of the river and which lie at distances varying from a few to as many as 40 or 50 miles from it. The country with which I am more immediately concerned in this report extends from Fundu at the southern end to the confluence of the Wira with the main river at the northern end, a distance of approximately 400 miles. Taking the average width of the valley to be 50 miles, the area thus included is at least 20,000 square miles. The Luangwa is fed by a large number of tributaries which enter it more or less at right-angles; but whereas many of those on the right side are large and permanent streams, those on the eastern bank, with one exception, flow only in the rains, and then only assume perceptible volume when carrying flood water. In general the floor of the valley is level and is covered to a very large extent by 'mopani' bush which becomes waterlogged, if not actually flooded, during the rains, and in which the grass is short and of scanty growth. This mopani bush usually ends rather abruptly at some distance from the streams and is replaced by more or less open country covered by dense and luxuriant growths of grass. As it is only in these situations that the soil repays cultivation, the villages, which are small collections of from 20 to 200 inhabitants, are found strung along the courses of the larger streams. This is more particularly the case on the eastern

side of the Luangwa, as it is only in the beds of the larger streams that water can be obtained by digging in the dry season. It may be noted that at this time of the year the Luangwa itself dries up in stretches in the upper reaches.

The mean altitude of the area under discussion may be taken as about 2,300 to 2,500 feet, so that the meteorological conditions are much more tropical than in the other portions of Northern Rhodesia. At Nawalia, which is about centrally placed at an altitude of about 2,100 feet, the mean temperature varied from 67° F. in July to 87° F. in November, and the relative humidity from 31 per cent. in September and October to 78 per cent. in January. The rain-fall averages about 40 inches and is spread over the six months, November to April, though the bulk is precipitated during the first three months of the year.

II. DISTRIBUTION OF GAME AND FLY

Game is both extremely abundant and varied throughout the whole of the valley. In the rains it ranges through the whole of the area, but in the dry season, more particularly after the grass has been burnt (July to September), it tends to collect along the courses of the streams where the grazing is better than in the mopani and where alone a supply of water is assured. At this time of the year it is not uncommon to find a relatively large animal population in fairly close proximity to the villages, as many of the species, e.g., waterbuck, roan, eland and bushbuck, are fond of feeding in the old gardens. I think this point should be emphasised, as it was demonstrated at Nawalia that waterbuck and bushbuck, amongst others, were infected by *Trypanosoma rhodesiense*, the first-mentioned to the extent of between 17·8 per cent. and 25 per cent. of the total number examined. It may here be noted that in the immediate neighbourhood of some villages in Kambombo's country which have suffered severely from sleeping sickness, waterbuck are extremely plentiful. There are grounds, therefore, for regarding this species of buck with particular suspicion.

Fly. *Glossina morsitans* is to be found throughout the whole of the valley and has been observed to show much the same seasonal variation as the game, i.e., in the dry season it is abundant near

the streams and hence near the villages, but after the rains have commenced tends to become more uniformly scattered over the country as a whole. This peculiarity has been observed in other parts of Africa. Owing to the fact that the villages are, as a rule, built in clearings and are surrounded by gardens, it is unusual for the fly to invade them, though an occasional specimen may follow the natives in. If so, it soon disappears again.

III. HISTORY OF SLEEPING SICKNESS

Attention was first drawn to the occurrence of human trypanosomiasis in the valley by the diagnosis of the disease in several Europeans about the year 1909. Investigations commenced then and carried out in the succeeding years showed that it was scattered over the whole of the area though the number of cases found was comparatively small. They demonstrated further that the infection displayed no tendency to assume epidemic proportions. Writing at the end of 1912, the Principal Medical Officer stated: 'In the light of recent knowledge as to the presence of the necessary factors and apparently very suitable conditions for the production of an epidemic, it is difficult to understand why after four years there should be no evidence that such is likely to occur. It can be most reasonably concluded that there is some unknown but necessary factor wanting or some inhibitory influence present, i.e., that the disease is an old one and that there may be a certain immunity present which is limiting its spread.' This is in strict accordance with the native evidence, for those I have questioned have always consistently maintained that they have always known of the existence of the disease as far back as their memories carry, in some cases a matter of seventy years or so. They state that it cropped up only as isolated cases which were drastically disposed of in some localities. Amongst the Bawisa and Bansenga the terms 'Chilotera' and 'Nyamakazi' are used to denote the symptom-complex of fever, oedemata of the extremities, protuberant abdomen, diarrhoea and emaciation, all commonly observed in sleeping sickness, though, of course, they do not associate the disease with tsetse flies. My own belief coincides with that of the natives, that the disease is an old one and not of recent introduction.

IV. INCIDENCE OF THE DISEASE

The observations with which I am now about to deal are all, with few exceptions, derived from data collected by me during 1913 and the early part of 1914, and after the conclusion of the war, from 1920 onwards. In this work I have relied on gland palpation and puncture as the means of diagnosis and only departed from it in exceptional cases and for special reasons, e.g., where a native's illness appeared to be due clinically to sleeping sickness but no palpable glands were found. Under these conditions, I claim that the various sets of figures may be properly compared and that they give a real indication of the relative incidence of the disease in the different years and in the same localities. I do not claim, however, that they indicate the absolute incidence of the disease. While I am of the opinion that gland palpation and puncture is the quickest and only feasible method of examining large bodies of natives, and that the great majority of cases will be found by its employment, I admit that some cases, particularly those in the very early stages of the infection, before the lymphatic glands have hypertrophied and in which no other symptoms are present, will be missed.

As mentioned earlier, the disease is very widely distributed and has been found in the Petauke, Serenje, Fort Jameson, Lundazi, Mpika and Chinsali portions of the valley. Further it is, as a general rule, comparatively rare, as the following figures will demonstrate :—

| Year examined | District | Natives examined | Cases | % infected |
|---------------|----------------------|------------------|-----------|-------------|
| 1913 | Mpika | 2,613 | 2 | 0·08 |
| | Lundazi | 13,100 | 9 | 0·07 |
| | Chinsali | 1,465 | 4 | 0·26 |
| 1914 | Serenje | 1,981 | 2 | 0·10 |
| | Petauke | 3,654 | 2 | 0·05 |
| | | <u>23,113</u> | <u>19</u> | <u>0·08</u> |
| 1921 | Lundazi, Part | 3,723 | 1 | 0·03 |
| | Mpika " | 1,311 | 0 | 0·00 |
| | Chinsali " | 913 | 1 | 0·11 |
| | | <u>5,947</u> | <u>2</u> | <u>0·03</u> |

As illustrating the general tendency for the disease to remain stationary over a period of years the following figures may be quoted. In every instance they are for the same villages in the respective years.

| District | Year | Natives examined | Cases | Year | Natives examined | Cases |
|-----------------|------|------------------|-------|------|------------------|-------|
| Lundazi | 1913 | 2,812 | 0 | 1921 | 3,530 | 1 |
| Mpika | | 1,233 | 0 | | 1,261 | 0 |
| Chinsali | | 776 | 3 | | 913 | 1 |

A further example may be quoted from Dr. May's investigations in one particular area in the Petauke Sub-District where, in the three successive years 1910, 1911, and 1912 respectively, 7, 7, and 3 cases were found.

It is difficult, in the absence of a complete census and the registration of births and deaths, to estimate not alone the general death rate amongst these natives but also that more specifically due to sleeping sickness. The following calculations, however, may be given. At the end of 1912, Dr. May computed the adult death rate in a portion of the Petauke Sub-District to be 28 per 1,000 and the incidence of sleeping sickness to be 8 per 1,000, also amongst the adults only. In 1913 I made similar enquiries as carefully as was possible in the valley portion of the Mpika Sub-District and estimated the general death rate for the year 1912-1913, exclusive of accidents, to be 23·7 per 1,000, the adult rate to be 47·7 per 1,000 and the incidence of sleeping sickness to be 3 per 1,000 of the whole population seen. Speaking of the valley generally, I should be inclined to say that under ordinary circumstances the incidence of the disease is not in excess of 3 to 4 per 1,000 of the total population per annum, though of course it may be exceeded temporarily in those localities in which exacerbations of the infection occur (for example, the estimated death rate for 1920-21 of 25 per 1,000 in the countries of chiefs Tembwe and Kambombo).

A very striking feature of the infection is the extraordinarily sporadic manner in which it is found. Not only are the cases found in villages widely separated but they are also usually found occurring singly, and cases in the same village may be separated by an interval of years. Examples of this are given in the following tables, and it should be noted that approximately the same number of natives were examined on the various occasions.

| Village | Wallace, 1912 | Kinghorn, 1913 |
|--------------------|---------------|----------------|
| Mumamba | 1 | 0 |
| Daroba | 1 | 0 |
| Chuni | 0 | 1 |
| Mkasanga | 0 | 1 |
| Chombero | 0 | 1 |
| Kundawamawe | 0 | 1 |
| Temba | 0 | 1 |
| Chinyondo | 0 | 1 |
| Luchenga | 0 | 1 |
| Kampuzunga | 0 | 1 |
| Mulumgu | 0 | 1 |

| Village | 1920 | 1921 | 1922 | 1924 | 1925 |
|----------------------|------|------|------|------|------|
| Tembwe Virizi | 0 | 2 | 1 | 0 | 0 |
| Katangalika | 0 | 1 | 0 | 0 | 0 |
| Ng'anjo | 1 | 0 | 0 | 0 | 0 |
| Mwimba | 0 | 1 | 0 | 0 | 0 |
| Kajumba... .. | 1 | 1 | 0 | 1 | 0 |
| Kambombo | 0 | 1 | 0 | 0 | 0 |
| Chizonde... .. | 0 | 1 | 0 | 0 | 0 |
| Kambwiri | 1 | 0 | 0 | 0 | 0 |
| Kazembe... .. | 0 | 0 | 1 | 0 | 0 |
| Dungulungu | 0 | 1 | 0 | 0 | 0 |
| Chama | 1 | 1 | 0 | 0 | 0 |
| Kawanda... .. | 1 | 1 | 1 | 0 | 0 |
| Kapalakonje | 1 | 0 | 0 | 0 | 0 |
| Chitimbe... .. | 1 | 1 | 1 | 0 | 0 |
| Nyika | 2 | 1 | 0 | 0 | 0 |
| Luambo | 2 | 0 | 1 | 0 | 0 |
| Chileta | 0 | 0 | 1 | 0 | 0 |
| Hunga | 1 | 2 | 0 | 0 | 0 |
| Buli | 1 | 0 | 0 | 0 | 0 |
| Chitukula | 1 | 0 | 0 | 0 | 0 |
| Chiruarua | 2 | 0 | 0 | 0 | 0 |
| Zowole | 1 | 0 | 0 | 0 | 0 |
| Mtonya | 1 | 0 | 0 | 0 | 0 |
| Mkunguwe | 1 | 0 | 0 | 0 | 0 |
| Luchenga | 0 | 0 | 2 | 0 | 1 |
| Kapotwe | 0 | 1 | 0 | 0 | 0 |
| Makondola | 1 | 0 | 2 | 0 | 0 |
| Kakuni | 0 | 0 | 2 | 0 | 0 |
| Marunga | 1 | 0 | 0 | 0 | 0 |

How is this peculiar distribution and incidence to be explained ? As is well known, some observers, chiefly the Germans, maintain that there are two distinct trypanosomes, *brucei* and *rhodesiense*, existing side by side in tropical Africa, which are indistinguishable except for the fact that *T. rhodesiense* is capable of infecting man while *T. brucei* is not, but is restricted to game and stock. The other school, chiefly British, maintain that the two trypanosomes in question are identical ; that it is essentially a parasite of game ; and that man is ordinarily resistant to infection, though this may occur. Exactly what conditions are necessary before man does become infected are uncertain. The chief arguments of those who favour the non-identity hypothesis are : (1) Dr. Taute's experiments, and (2) the geographical argument, that in many localities where *T. brucei* is found cases of sleeping sickness have never been diagnosed. With reference to the first of these, I think the experiments may be held quite permissibly to prove the truth of the contention that man is naturally resistant to infection by *T. brucei vel rhodesiense* and that, in any event, they are not sufficiently extensive to prove indisputably the truth of the negative statement that the human and game trypanosomes are not identical. As regards the geographical argument, I am not aware that the localities usually cited have been thoroughly and repeatedly examined over a period of years, and that, at the same time, such important factors as the abundance of fly and game, the percentages of both harbouring *T. brucei*, *sensu strictu*, and the closeness of contact existing between them and the native population have been taken into consideration. Without departing from the confines of Northern Rhodesia, however, it appears to me to be a difficult matter to explain, on the assumption that human cases of the disease are invariably due to a specific human as distinct from the ordinary game trypanosome, the occurrence of one European and two or three native cases in the western part of the Serenje sub-district with an interval of years between them, more particularly as the examinations of the suspected area carried out by Dr. Masters prior to 1912, by Dr. Ellacombe in 1912, and again by Dr. Powell in 1920, gave negative results as far as the indigenous population was concerned. And further, it is peculiar that in the other area in this country in which the disease has been found

to occur much as it does in the Luangwa valley the same conditions of an abundance of game and fly co-exist in close contact with the natives. I refer to the focus in the south-western corner of the Ndola sub-district. Prof. Kleine, one of the protagonists of the non-identity theory, admitted in conversation that the local game was susceptible to infection by *T. rhodesiense*, *sensu strictu*, and this being the case it follows that it would only be a matter of time until this parasite was widely distributed amongst the fauna of the valley and one would then expect to find human cases to be both more numerous and more uniformly spread over the country as a whole. As pointed out above, there is a concentration of both the game and the fly in the vicinity of the villages in the dry season, and while a certain amount of evidence exists to show that the risks of infection are then greater it is not extensive enough to permit of any dogmatic statement on the point. I believe, personally, that the sporadic appearance and erratic distribution of the infection as it is generally seen and has been seen since 1909, with no tendency to assume epidemic proportions, is more satisfactorily explained by the theory that the human and game trypanosomes are one and the same parasite and that man is ordinarily resistant to infection by it than by the theory that the human and game trypanosomes are distinct entities.

While the normal incidence of the infection is as shown above, exacerbations have been observed from time to time in localized areas of the valley, but these, after the lapse of a few years, have always ended spontaneously and the disease has then reverted to what may be termed its equilibrium. The most pronounced of these has been the one which started early in 1918, in the contiguous territories of the three chiefs Chikwa, Tembwe and Kambombo towards the Northern end of the Lundazi sub-district. Of the three, Kambombo suffered most. Thus writing in May, 1920, the Native Commissioner, Lundazi, reported that since the commencement of this outbreak some 131 deaths had occurred to date from what appeared to be sleeping sickness, amongst an adult population estimated at 1,455 on March 31st, 1920, in the villages under that chief. It may be noted that at the end of 1917, or beginning of 1918, plague appeared in this same area and was not stamped out until 1919. This coincidence was probably quite fortuitous except that many of the

villages were burnt and the natives forced to build temporary quarters in the bush. This, of course, brought them into closer and more continuous contact with fly than is ordinarily the rule, and to some extent an increase in the number of cases of sleeping sickness may be ascribed to this cause, but it cannot have been of general application, as those villages which suffered most severely from sleeping sickness were not destroyed. In 1920, and the succeeding years, I examined and re-examined the natives in this particular area with the following results :—

| Year | Natives examined | Cases | % infection |
|--------------|------------------|-------|-------------|
| 1920 | 5,756 | 25 | 0·43 |
| 1921 | 5,634 | 18 | 0·32 |
| 1922 | 5,317 | 9 | 0·17 |
| 1924 | 4,347 | 1 | 0·02 |
| 1925 | 4,301 | 2 | 0·04 |
| Compare 1913 | 5,122 | 3 | 0·06 |

Or if we consider the three sets of natives separately we have the following percentages of infection in the various years :—

| Year | PERCENTAGE INFECTION | | |
|------|----------------------|--------|----------|
| | Chikwa | Tembwe | Kambombo |
| 1920 | 0·25 | 0·73 | 0·36 |
| 1921 | 0·15 | 0·27 | 0·57 |
| 1922 | 0·22 | 0·12 | 0·15 |
| 1924 | 0·00 | 0·00 | 0·06 |
| 1925 | 0·07 | 0·00 | 0·06 |

It should be noted that these percentages only represent the incidence of the disease at the actual time of examination, and, while under 'normal' conditions they might afford an approximate idea of its frequency, they are much too low for 'Epidemic' conditions. Thus in 1921, by tracing the causes of deaths in the interval between that and the former visit, I estimated that the then rate of infection was about 2.5 per cent. of the whole population per annum. I am not fully satisfied that the 1924 figures give anything like a true indication of the percentage of infection in that year, as I saw about a thousand fewer natives than in former trips, and amongst those who evaded examination cases may have existed; but it may be noted that the deaths between 1922 and 1924, which could be attributed to sleeping sickness were comparatively few; hence it seems reasonable to conclude that this localized 'semi-epidemic' is behaving like the others and that the infection is becoming stabilised again. That this conclusion is essentially true is borne out by the results of the trip I have just finished through this area. As will be seen above, 4,301 natives were seen and 2 cases of the disease diagnosed, a percentage of 0.04. In the six months which elapsed between my 1924 and 1925 trips some nine deaths, which might possibly have been due to sleeping sickness, occurred, so that it would appear that the annual incidence of the disease in this particular area is now not in excess of 5 per 1,000 of the total population.

I am of the opinion that these epidemics are due largely to the superimposition of a man-fly-man cycle of transmission of the parasite on the more ordinary game-fly-man cycle and the occurrence of this is often hastened by some of the habits of these natives. For instance, cases may be carried from one village to another owing to the custom of returning to the original home when a native falls sick. Again, during the rains the villages split up, each family living in the middle of its gardens in order to protect them from the depredations of monkeys and elephants, and cases of infection in man and wife and parent and child have been observed under these conditions. Further, the habit of growing crops between the huts in the villages themselves increases the liability of their invasion by fly with the consequent danger of their becoming infected should a case of sleeping sickness exist.

V. THE DISEASE IN HUMAN BEINGS

The incubation period is short, probably between one and two weeks. At first the only symptom is fever with its concomitants, followed by enlargement of the lymphatic glands, particularly in the basal portion of the posterior neck triangles, progressive emaciation, oedemata of the extremities and face, protuberant abdomen, diarrhoea, anaemia, muscular tremors, inco-ordination, mental hebetude and somnolence deepening into coma. In untreated cases, death is the inevitable result and the whole duration of the infection is short, on the average from about three to six months. An occasional case may live for ten or twelve months, though this is very exceptional, and only one, in my experience, has ever exceeded this period.

This native, Chimwila, was found to be infected on the 18th November, 1920, and then gave a history of having been ill only one month, complaining of headache. This was probably an underestimate as his neck glands were markedly enlarged. He was seen again on the 9th May, 1921, and appeared to be in perfect health saying himself that he was not sick. His neck glands were now smaller though the juice still contained parasites. His condition remained the same on the 11th March, 1922, and trypanosomes were still found in the gland juice. He was then sent to Prof. Kleine for treatment with Bayer 205, i.e., sixteen months after he had been found to be infected and probably eighteen at least after he contracted the disease. Prof. Kleine states in one of his reports that Chimwila appeared to be physically in good health on his arrival but that some mental dullness was noticed. After receiving treatment he returned to his village later in 1922 and was apparently quite well when last seen in March, 1925. No glands were palpable and no parasites were found in the blood.

The disease is very decidedly one of adolescence and adult life. I have never seen a case in a young child, and only comparatively rarely under the age of about fifteen. This is well brought out in the following tables from two independent sources, and in view of the great difficulty of estimating at all accurately the ages of natives and the part the personal equation must play in such estimations, the general agreement of the two sets of figures is very striking.

| Age | KINGHORN | | FISCHER | |
|---------|----------|-------|---------|-------|
| | Cases | % | Cases | % |
| 6-15 | 5 | 6.66 | 1 | 3.57 |
| 16-25 | 20 | 26.66 | 9 | 32.14 |
| 26-35 | 27 | 36.00 | 11 | 39.28 |
| 36-45 | 17 | 26.66 | 5 | 17.85 |
| 46-55 | 5 | 6.66 | 1 | 3.57 |
| Over 55 | 1 | 1.33 | 1 | 3.57 |
| | 75 | 99.97 | 28 | 99.98 |

It will be noticed from these tables that there is a steadily increasing susceptibility to infection from the earlier ages to about the 35th year and that thereafter the decline in susceptibility is as equally and steadily marked ; further, that from 85 to 89 per cent. of all the cases occur in the age group 16 to 45. These remarks apply with equal force to the two sexes considered separately, as shown below.

| Age | MALES | | FEMALES | |
|---------|-------|-------|---------|-------|
| | Cases | % | Cases | % |
| 6-15 | 4 | 8.88 | 1 | 3.33 |
| 16-25 | 11 | 24.44 | 9 | 30.00 |
| 26-35 | 15 | 33.33 | 12 | 40.00 |
| 36-45 | 12 | 26.66 | 5 | 16.66 |
| 46-55 | 3 | 6.66 | 2 | 6.66 |
| Over 55 | 0 | 0.00 | 1 | 3.33 |
| | 45 | 99.97 | 30 | 99.98 |

This table also brings out the fact that the disease is commoner in men than in women, the proportion being as 3 : 2, and this is corroborated by an analysis of Dr. Fischer's cases, which gives a proportion of $3\frac{1}{2}$ males to 2 females. Probably the greater number of male cases may be accounted for on the basis that the men, on the whole, spend more time in the bush, hunting, collecting honey, poles for building, reeds and other material for mat and basket-making, and so on, than the women do in their daily trips to collect firewood. It is more difficult to explain why, approximately after the age of puberty is passed, the susceptibility to infection should increase so perceptibly and increase in an ascending curve to the age of thirty-five and then decrease steadily. Babies and very young children are always carried by their mothers in a calico or sling and are thus fairly effectively protected, but the older children who also usually accompany their mothers wherever they go are naked and unprotected. The older children, aged from ten to twelve onwards, are, at best, very scantily dressed and have to assist their parents in the various duties outlined above. They are thus frequently exposed to the risk of infection and yet it is only very rarely that this occurs. It may be argued that the discrepancies in the age-incidence of the disease are more apparent than real and that if the actual age distribution of the whole population could be plotted, these would become obvious, but in the absence of an accurate census this is impossible. I am, however, inclined to doubt this. Certainly as between children and adults it does not apply, as it may be said generally that the number of children in a village will about equal the number of adults. Thus an analysis of the two groups in the villages under Chikwa, Tembwe and Kambombo, gives 2,741 children to 3,015 adults. I am convinced that the varying rates of infection amongst the different age-groups cannot be explained wholly and satisfactorily on the assumption that they are in direct ratio to the risks of infection run by the respective groups, though it is difficult to suggest other factors which may influence the occurrence of the disease in the light of our present knowledge. It may here be pointed out that hunger with its consequent lowering of vitality does not increase susceptibility to infection. In this area the 1923-24 crops were bad, with the result that, towards the end of 1924, acute hunger,

amounting in some villages to actual starvation, became apparent, and several deaths from this cause were reported. The results were particularly noticeable in Chikwa's country, where all of the natives showed emaciation varying from slight to extreme, yet only one case of sleeping sickness was found, and only five deaths from what may have been sleeping sickness were reported amongst 1,400 natives.

There is no evidence that acquired immunity to the infection is found in man though, as stated above, I am of the opinion that he is naturally very resistant to it.

VI. TREATMENT WITH 'BAYER 205'

Early in 1922, a commission composed of Prof. Kleine and Dr. Fischer came out to this country to try the effects of the drug known as 'Bayer 205' on human trypanosomiasis and a camp was established in the Luangwa valley at Ndombo, about thirty-five miles east of Mpika. In all, 38 cases were treated, and of these one man went later to Southern Rhodesia and has not been traced, five are still alive, and the remaining 32 are dead. Five of the deaths occurred at the Commission's camp from intercurrent affections and the other 27 in the villages at varying periods after the parents had returned there on leaving Ndombo in October, 1922. The following is the complete list :—

| Name | Sex | Age | Date of death | Duration of life after treatment | Remarks |
|-----------------------|-----|-----|----------------|----------------------------------|----------|
| 1. Pitala | M | 25 | February, 1924 | 16 months | |
| 2. Yatula | F | 30 | — | — | Alive |
| 3. Chimwila | M | 35 | — | — | Alive |
| 4. Chizilicho | M | 35 | March, 1924 | 17 months | |
| 5. Isake | M | 15 | July, 1923 | 9 " | |
| 6. Chilukupata | M | 25 | April, 1924 | 17 " | |
| 7. Mofati... .. | M | 35 | ? | ? | Untraced |
| 8. Lamek | M | 16 | — | — | Alive |
| 9. Samuel | M | 16 | February, 1924 | 16 months | |
| 10. Tadeyu | M | 27 | February, 1924 | 16 months | |

| Name | Sex | Age | Date of death | Duration of life after treatment | Remarks |
|------------------------|-----|-----|--|----------------------------------|-------------------|
| 11. Kajawa | M | 38 | — | — | Alive |
| 12. Vioka | F | 22 | October, 1923 | 12 months | |
| 13. Mateyo | M | 35 | February, 1923 | 4 months | |
| 14. Chifundulwa | M | 60 | — | — | Alive |
| 15. Lasalu... .. | M | 25 | February, 1924 | 16 months | |
| 16. Vilauli | M | 38 | July, 1924 | 19 months | |
| 17. Malita | F | 22 | November, 1923 | 13 „ | |
| 18. Mwipi | M | 12 | December, 1923 | 14 „ | |
| 19. Murere | F | 45 | October, 1923 | 12 „ | |
| 20. Nderema | F | 40 | October, 1923 | 12 „ | |
| 21. Ntanda | F | 27 | October, 1923 | 12 „ | |
| 22. Yumba | F | 45 | November, 1923 | 13 „ | |
| 23. Ndabeya | F | 54 | April, 1923 | 6 „ | |
| 24. Mage | F | 26 | February, 1924 | 16 „ | |
| 25. Marata | M | 28 | July, 1923 | 9 „ | |
| 26. Mapulanga | M | 35 | February, 1923 | 5 „ | |
| 27. Kabrieni | M | 18 | November, 1923 | 13 „ | |
| 28. Chikoti | M | 35 | October, 1923 | 12 „ | |
| 29. Kamcheba | F | 32 | October, 1923 | 12 „ | |
| 30. Zakeyo... .. | M | 35 | October, 1923 | 12 „ | |
| 31. Wayilipa | F | 35 | October, 1923 | 12 „ | • |
| 32. Sabeta | F | 20 | October, 1923 | | Died on road home |
| 33. Thomas | M | 17 | February, 1924 | 16 months | |
| 34. Thomas | M | | | | |
| 35. Ngoza | F | | | | |
| 36. Chiweza | M | | died at Ndomba from intercurrent disease | | |
| 37. Tepatepa | M | | | | |
| 38. Puntayila | M | | | | |

As will be noticed, the cases were of both sexes and of all ages and it may be added that they were in various stages of the disease from early to late. With regard to the cases which died in the villages, the native evidence is that at varying periods after they had returned they started to become ill again and presented the ordinary symptoms of sleeping sickness, e.g., progressive emaciation, oedemata, and so on. This applies equally to the eight cases which died in October, 1923, but there is a possibility that in these death was hastened by influenza of which an epidemic swept through the valley in August and September of that year. This reappearance of symptoms must be regarded as due to relapses of the original, and not to fresh infection. In the treatment of sleeping sickness the greatest drawback to success is the difficulty of attacking effectively the parasites in the cerebro-spinal fluid, and evidence of this with 'Bayer 205' is found in the reports of the German Commission. Thus it is stated that of twenty-one patients examined by lumbar puncture before their discharge from the Ndombo Camp in October, 1922, eight were found to harbour trypanosomes. Under such circumstances it would only be a matter of time until the trypanosomes re-invaded the blood stream and set up symptoms of the disease. The only definite result claimed by the Commission for 'Bayer 205' is that by its use it is possible to sterilise the blood for a long period even in those cases which are not clinically cured ; and while this claim seems to be established I question, in view of the demonstrated fact that the bulk of the cases did ultimately relapse, whether there are any substantial grounds for stating that ' if in districts infected with sleeping sickness all suspected natives receive treatment . . . the source of infection for the tsetse flies will gradually disappear and in time the disease must die.' It is assumed that man is the only reservoir and that the game is negligible. This I cannot admit.

The routine method of treatment adopted by the Commission was three subcutaneous or intravenous injections of 1.2 gm. in normal saline at intervals of ten and eighteen days, though those cases in which the parasites persisted in the blood and cerebro-spinal fluid received a fourth and fifth injection. It is apparent, therefore, that a dosage of from 3.6 to 6 grammes is insufficient to cure the majority of cases of *T. rhodesiense* infections in natives. Better

results might, of course, follow the adoption of an increased dosage up to 10 or 12 grammes as has been advised by some experienced workers, but at the price of 6/6 a gramme it is questionable whether the expenditure of £3/5/- to £4 per head on drugs alone would be legitimate, in the light of our present knowledge, on any very wide scale, more particularly as reports from the Congo would indicate that in tryparsamide we possess an equally, if not more, efficacious drug which possesses the advantage of being cheaper. It is of interest to note that in these reports it is said that the results of treatment there with 'Bayer 205' had been 'frankly disappointing.'

I saw and examined the five cases which are still alive in August, 1924, and again last month. All of them appeared to be in perfect health, presented no signs or symptoms of sleeping sickness, and showed no parasites in the blood stream. It was not possible to perform lumbar puncture. I believe, therefore, that these natives may now be regarded as being definite cures. Disregarding the five cases which died from intercurrent disease and the one which has not been traced, this represents a percentage of 15.6 cured. In view of the fact that all previous methods of treatment for the Rhodesian type of the disease have been failures, this must be admitted to be a considerable advance, but these results do not justify the very optimistic claims which are still being made for this drug.

VII. PROPHYLAXIS

It is a definite fact, which must be recognised, that no active assistance can be expected from these natives for any measures designed either to combat a specific infection, or generally to improve the sanitation of the villages. For some years movement into and out of the valley was prohibited, and even though the sleeping sickness regulations have fallen into comparative desuetude very few Europeans travel there now. Thus to a marked degree the natives have remained under the influence of their ancient tribal beliefs and treat European ideas of the etiology of disease and the methods to be adopted in treating them with undoubted, if unexpressed, disbelief. The general attitude, therefore, becomes one of passive resistance which can only be overcome by a certain

amount of compulsion, and when force has to be employed the results are usually unsatisfactory. Not only does the native revert to his own ideas and habits as soon as he thinks he can safely do so, but there is also a tendency to evade actively the application of disagreeable rules and regulations by running away and hiding as soon as an official appears in his district. Of this I have had personal experience. This becomes more pronounced when the results of European regulations and treatment are unsatisfactory, as it must be admitted they have so far been with particular reference to sleeping sickness. My experience is that the few cures are overlooked and attention is concentrated on the failures, and that indeed the natives really believe that the deaths have been caused by the use of hypodermic needles. In time, and with the increase of education, this general attitude may be modified but it will necessarily be a very slow process, and in the interval it is not apparent that much can be done. Something might be done to hasten this, by arrangement with the various missions having schools in the valley, if the teachers were given instruction in the essential facts of the etiology of sleeping sickness and other infections, told to explain these facts to their classes at frequent intervals, and to urge patients to go to hospital for treatment. No immediate results could be expected in view of the ingrained instinct of the native to return to his home as soon as he falls sick, and in view of the widespread aversion to going into hospital. I think, however, it would be a step in the right direction.

In view of the facts, which I think have been brought out earlier, that sleeping sickness ordinarily is one of the less important causes of death in the valley and that in general it occurs only in widely-scattered, sporadic cases, I am inclined to doubt whether it is incumbent on the Government to provide special facilities for treatment beyond those which already exist in the various hospitals. When the enormous area of the valley is recalled it is obvious that to do so would entail, if success is to be obtained, the appointment of at least three special medical officers with hospital and staffs, and the expense of this would, of course, be very large. Success could only be anticipated if the theory is true that *T. rhodesiense* is a parasite of human beings alone, that man is the sole reservoir and that the game plays no part in the perpetuation of the infection.

If so, then it appears to me that the obligation to deal with the disease by special measures is greatly strengthened. If, however, it is correct that the game and human parasites are identical, or alternatively that the game may act as a reservoir for a specific human trypanosome, then success in combating sleeping sickness can only be assured by simultaneously segregating and treating all the human cases of the disease and killing off the whole animal fauna. This is not a practicable proposition in the valley.

In the event of the occurrence of one of the localized epidemics it would, I think, be advisable to institute local treatment, as I believe that in these man does play a part as a direct reservoir of infection and it would be a matter of some importance to break the man-fly-man cycle.

I also think that it would be advisable to keep on the statute book the Sleeping Sickness Regulations, not with any idea of interfering with the natives, but chiefly for the power they confer of regulating the movements of Europeans. If all the regulations were rescinded, there would probably be an influx of professional hunters in pursuit of elephant, and the possible occurrence of cases amongst them might entail an unnecessary expense on the Government.

Lundazi, N. Rhodesia,
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THE DESTRUCTION OF ASCARIS EGGS

BY

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Most investigators are agreed that *Ascaris* eggs have strong powers of resistance to chemical fluids of various kinds. I also have tested the resistance of *Ascaris* eggs containing embryos by soaking them in various disinfectants, acid or alkaline, and have found that in every experiment they retained for many days the ability to infect.

I found, however, that *Ascaris* eggs were easily destroyed by heat. In September, 1921, I poured hot water at a temperature of 100° C. over *Ascaris* eggs collected from human dejecta and found that the eggs lost their power of development. In November of the same year I noticed that *Ascaris* eggs containing embryos, after hot water had been poured on them, lost the ability to infect.

Having ascertained that *Ascaris* eggs were instantly destroyed by water at 100° C., I attempted to discover how long they would remain alive in water at varying degrees of temperature. These tests, however, failed on account of the difficulty of finding any effective means of immersing in hot water *Ascaris* eggs dispersed among human dejecta. Again, if eggs mingled with human dejecta are dispersed in boiling water, it is not always possible to get them out at once.

At length, a method was discovered which seemed to me entirely effective. The following experiments were then carried out.

Method: In testing the resistance of *Ascaris* eggs to heat, I employed matches in order to prevent the dispersal of the eggs. The human dejecta were washed in water and the sediment obtained by centrifuging was smeared on the matches to half their length, and left to dry. Then each match, held with the forceps, was soaked for the desired period in hot water at varying temperatures. They were then put into cold water to cool. The matches were then subjected to observation.

The observations were carried out in three ways, (1) staining; (2) development; (3) infection.

(1) I use Sudan III staining for the purpose of distinguishing between live and dead *Ascaris* eggs. Presuming that fatty

degeneration might possibly occur in the liver of a patient infected with *Ascaris*, we applied Sudan III staining to the liver of an animal which was experimentally infected with *Ascaris*. We found that *Ascaris* larvae in the liver were distinctly stained by Sudan III staining, and that living larvae separated from the liver or lungs could be similarly stained. The red-stained granules of 'fat-corpuscles,' as I provisionally call them, gradually decrease in size as the size of the larva increases at each stage of its development.

With the object of making clear the true nature of the 'fat-corpuscles' of the *Ascaris* larva, I tried to ascertain if there were any substance in *Ascaris* eggs which could be stained with Sudan III. It was necessary to make sections for the staining of the eggs. The *Ascaris* eggs, collected from human dejecta, were embedded in gelatine and cut into thin sections with the freezing microtome. They were then stained with Sudan III. By this means the staining of eggs was obtained, but not of the albuminous membrane. I further noticed that fat-corpuscles were abundant in the eggs.

When I applied Sudan staining to *Ascaris* eggs collected from human dejecta, I found that unfertilised eggs were stained with red granular spots, while healthy fertilised eggs were not stained at all. Therefore, presuming that *Ascaris* eggs might be made stainable by killing them, I applied Sudan III staining to eggs killed by boiling water. As I expected, all the eggs were seen under the microscope with fine red colouring.

From the results of my repeated experiments, I conclude that the fat-corpuscles are produced in the blastomeres as the egg-cells grow and segment, and that gradually these fat-corpuscles gather much more in the hypoblastic than in the epiblastic cells; thus very young U-shaped embryos are full of fat-corpuscles from head to tail; but these fat-corpuscles are ranged along the intestine of the worms as they grow. We consider, therefore, that the above-mentioned fat-corpuscles may be the yolk.

Having found, therefore, that (a) healthy fertilised eggs are unstainable with Sudan III, that (b) unfertilised eggs are stainable, and that (c) fertilised eggs become stainable with that dye if hot water is poured over them, we used Sudan III for the study of *Ascaris* eggs after immersion in hot water at temperatures varying from 40° C. to boiling point, and for periods varying from one second

to an hour. We found that *Ascaris* eggs become stainable with Sudan III after being immersed for one second in water at over 75° C., almost all become stainable after being immersed for ten seconds in water at 70° C., while in water at 65° C. they had to be immersed for over ten minutes before becoming stainable. In water at temperatures lower than 60° C. over one hour's immersion is not sufficient to render them stainable.

The above experiment was repeated twenty times with material from the same patient and from twenty-one others, but the results showed no great difference.

(2) In order to prove that *Ascaris* eggs which have become stainable are really dead, we cultivated, in 4 per cent. formalin solution, *Ascaris* eggs which had been immersed in water at varying temperatures for varying periods of time as in Experiment 1, and examined their developmental condition at the end of stated periods. This was repeated some thirty times with material from the same patient and from many others, but all the results were practically the same, namely, that all the eggs lost their power of development after being immersed in water at over 70° C. for one second; a few retained it after being dipped in water at 65° C. for one second; embryos developed from all eggs immersed for three seconds in water at 60° C., for 40 seconds in water at 55° C., and for thirty minutes in water at 50° C.; all eggs would develop into embryos after being soaked for one hour in water at temperatures lower than 45° C.

The difference between the results of Experiments 1 and 2 is noteworthy.

Ascaris eggs which have lost their power of development can be divided into two groups, one stainable with Sudan III, the other unstainable with that dye. The former show a morphological change (i.e., fatty degeneration and distortion of the eggs) after immersion in hot water; the latter do not differ from healthy eggs, and yet are in a state of suspended development. This condition of suspended development may continue for as long as twenty days or even longer, but if left alone for long they are likely to perish gradually.

Ascaris eggs are instantly killed by very hot water; by water at lower temperatures they are merely deprived of their ability to develop; and after being immersed in water at temperatures below 45° C. the eggs develop in the usual manner.

(3) As a result of Experiments 1 and 2 I could definitely fix the temperature at which hot water will destroy *Ascaris* eggs containing embryos capable of infection.

I cultivated for a month, in 4 per cent. formalin solution, *Ascaris* eggs collected from human dejecta. When matured, I placed them on matches and left them to dry until the following day. I then soaked these matches in hot water at various temperatures and fed mice with the eggs. After three days I examined the mice with a view to ascertaining whether liver, heart, or lungs were infected. I repeated this experiment many times with 515 mice and arrived finally at the following results.

The mature *Ascaris* eggs, cultivated in 4 per cent. formalin solution, lose their infecting power after remaining for one second in water at 70° C. or more; almost all lose it after remaining for one second in water at 65° C., for five seconds in water at 60° C., for forty seconds in water at 55° C., or for fifteen minutes in water at 50° C. Even after remaining for one hour, however, in water at temperatures below 45° C. the eggs did not lose their power to infect.

SUMMARY

A minute examination of the power of resistance to heat of *Ascaris* eggs by means of the above experiments has led to the conclusion that the method of destruction of *Ascaris* eggs by boiling water can be effected also with hot water at lower temperatures. Generally speaking, the ideal to be aimed at in disinfection is simplicity of method and rapidity of action.

To prevent *Ascaris* infection it is safest to kill the eggs by immersion in hot water at the temperature at which the egg content changes morphologically and becomes stainable with Sudan III; though the power of development and the power to infect may be destroyed by immersion in water at over 70° C. for one second, at 65° C. for two seconds, at 60° C. for five seconds, at 55° C. for fifty seconds and at 50° C. for forty-five minutes.

In conclusion, I wish to acknowledge my great indebtedness to Dr. S. Yoshida who kindly superintended my work and gave me all facilities for completing the present paper.

AN EXPERIMENTAL STUDY ON THE DEVELOPMENT OF THE DWARF TAPEWORM (*HYMENOLEPIS NANA*)

BY

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The course of infection and the development of the dwarf tapeworm have been much studied and discussed, especially as to whether this species has any intermediate host or not, but as yet no definite conclusion has been reached. I have studied this subject extensively, both in animals and in man.

From my experiments it appears that the eggs of this tapeworm, unlike those of others, can hatch and develop without any intermediate host.

ON THE DEVELOPMENT OF THE DWARF TAPEWORM IN ITS HOSTS

1. *From five hours to twenty-four hours after feeding.*

On examining mice which had swallowed dwarf tapeworm eggs five or six hours previously, I observed in their small intestines six-hooked larvae, all of which had emerged from the egg-shells. After ten hours the mice had larvae which had already entered into the villi in the upper part of the small intestine and from hour to hour, up to twenty to twenty-four hours, larvae penetrated the villi. These larvae were oval or round in shape, their surface was granulated and they had six hooks near one end.

2. *Three days after feeding.*

I found many larvae in the villi; their shape was the same as noticed above but they had increased in size. Most of them were somewhat pear-shaped. In the centre of their bodies the granules

became somewhat coarse and I observed some small calcareous corpuscles which reflected the light strongly, but as yet the larvae were not encysted.

3. *Four days after feeding.*

The six-hooked larvae, which had entered the villi, had increased much in size and became bean-shaped encysted larvae (*Cysticercus*), the space between the body of the larva and the cyst being filled with granular material. Some, still with six hooks at one end of the cyst, were moving in the villi.

In the centre of the body of the larvae was a ring of regularly arranged wedge-shaped hooks, which reflected the light strongly; but the roots of the hooks were not yet separated and the differentiation of the suckers was not clearly defined; however, by carefully adjusting the light, I could see some encysted larvae with two suckers in front and some with only one; and among the radiating fibres running from the caudal extremity of the encysted larva to the cyst, could be found irregularly-shaped calcareous corpuscles, some large, some small.

4. *Five days after feeding.*

Well developed encysted larvae had emerged from their cysts, left the villi, descended to the lower part of the small intestine and were actively moving about, extending and contracting themselves; some were in process of emergence from their cysts and some had already emerged, still having parts of the cysts attached at the rear. In these larvae I could see four suckers; a single ring of hooks, an excretory tube, running from the rear of the rostellum to the caudal extremity, and some irregularly-shaped calcareous corpuscles which reflected the light strongly.

5. *Eight days after feeding.*

The size has increased and segments are clearly seen, but there is no differentiation of the reproductive organs.

6. *Ten days after feeding.*

A gradual increase in size; the uteri being filled with what seemed to be primitive eggs, the structure of which was not very clear.

7. *Fourteen days after feeding.*

Growth is complete and the posterior gravid segments are so full of eggs that the testes are compressed and the excretory tubes obscure.

8. *Fifteen to nineteen days after feeding.*

Although there was some difference in the development of the dwarf tapeworms according to the host employed (mice, rats) they grew little by little, and about the seventeenth or eighteenth day, the experimental animals began to evacuate eggs in the faeces.

After eighteen days the last segments of the adult tapeworms become more slender, as the result of having discharged many eggs.

EXPERIMENTAL INFECTION IN MONKEYS

15 June, 1919. Two young male monkeys (one year and two months old), showing no tapeworms' eggs in their faeces, were obtained. I made one of the monkeys swallow many eggs of the dwarf tapeworm, but found no eggs on examining its faeces fifteen to sixteen days after the feeding; so I again made it swallow many eggs. It was killed seven days later; the contents and villi of its small intestine were closely examined, but no dwarf tapeworms were to be found.

26 July, 1919. The other monkey was fed with many dwarf tapeworms' eggs; it became gradually feeble, and died in about six days. Examination of its small intestine showed thirteen young dwarf tapeworms in the lower part, the shape and size being no different from those of the young dwarf tapeworms in the small intestine of the mice and rats.

SWALLOWING OF EGGS BY MAN

29 January, 1919. Taking great care not to destroy the eggs of the dwarf tapeworm, I washed them with water, put many of them into capsules and swallowed them myself.

As a control, a part of the same material was given to a rat and a white rat which seven days later proved to be infected, but in spite of having examined my own faecal matter several times, for fourteen or fifteen days after ingestion, I could not find any dwarf

tapeworm eggs. Then I tried to expel tapeworms from my intestine, but unsuccessfully. Afterwards I swallowed eggs three times but neither eggs nor worms were found in my faeces.

Fortunately, a girl four years old was available for experiment; she was strongly-built, well nourished and healthy. I carefully examined her faeces several times, and each time found a few eggs of *Ascaris* but none of the dwarf tapeworm. On 12 April, 1919, she swallowed many eggs of the dwarf tapeworm in capsules and afterwards her faeces were examined on many occasions. On the first of May, nineteen days later, I found a few eggs of the dwarf tapeworm, and upon expelling the worms from her small intestine, I secured ninety-seven adult dwarf tapeworms.

SUMMARY

1. The six-hooked larvae of the dwarf tapeworm, about ten hours after ingestion, penetrate the villi in the upper part of the small intestine and four days later become encysted larvae (*Cysticercus*). Five days after ingestion, well developed larvae emerge from their cysts and leave the villi.

2. After seven days, well developed young dwarf tapeworms have some segments at the end of the body.

3. After nine days, their reproductive organs become visible.

4. About fourteen days after ingestion, the segments are full of eggs, in each of which can be seen a six-hooked larva (*Onchosphere*).

5. After about seventeen days, the ripe eggs of the dwarf tapeworms are found in the faeces of the experimental animals.

6. These experiments show that dwarf tapeworm eggs which have been evacuated with the faeces of the host, are swallowed by animals or man with their food, and that the eggs hatch and the six-hooked larvae are liberated in the small intestines, and later enter the villi of the small intestines where they become encysted larvae; then emerging from the cysts they grow into young dwarf tapeworms and upon maturity evacuate the ripe eggs.

7. Therefore, without any intermediate host, the dwarf tapeworm can directly develop in the body of mice, rats, young monkeys, or man, especially children.

A SPOROZOON OF *PHLEBOTOMUS PAPATASII*

BY

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AND

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(*Received for publication 9 July, 1925*)

During a routine examination of 1,037 *Phlebotomus papatasi*, of which 939 were females, one female was found to contain oocysts. All the insects were collected in Jericho between April and June, 1925. The infected specimen was one of a number used for experimental purposes and dissected immediately after a feed on a laboratory assistant on 25th May, 1925.

On dissecting the head from the thorax a large number of small glistening bodies were seen to emerge from the thorax and these on examination proved to be sporocysts from a ruptured oocyst.

Further dissection revealed the fact that thorax and abdomen contained four ripe oocysts, one in the thorax and three in the abdomen. The oocysts were 130μ by 95μ in size and contained about a hundred sporocysts. Between the sporocysts were a number of round refractile bodies up to 8μ in diameter. The sporocysts varied in size from 21.4μ to 36.4μ in length by 15.7μ to 20μ in breadth and contained four to sixteen sporozoites and a residual body, from 3.6μ to 6.4μ in diameter, enclosed in a definite membrane. Apart from the residual body each sporocyst contained a number of small refractile granules lying apparently in the sporozoites.

In the sporocysts the sporozoites were seen to be actively motile, in some cases sufficiently so to cause the whole sporocyst to spin.

From the ruptured sporocysts sporozoites were seen emerging; each sporozoite was then observed to be lying in a membrane which when released from the sporocyst assumed the form of an elongated

spindle about 35μ in length and 6.4μ in breadth (figs. 6 to 8, and 12). The small refractile granules noted in the sporocyst were found to lie in the membrane outside the sporozoite, each membrane containing two to four granules.

The membranes, including the sporozoites, were all seen to be divided longitudinally by two fine lines (fig. 12). The sporozoites were actively motile within their membranes, constantly changing their shape and size by a series of contractile movements, so that it is difficult to give definite measurements. When fully stretched out the sporozoites were sickle-shaped with one end pointed and the other blunt, and then measured 34μ in length by 5μ in their thickest part (fig. 9). Each sporozoite contained a round nucleus.

By contracting and thus increasing their transverse diameter the sporozoites stretched the enclosing membrane and created a gap between the two longitudinal lines of the membrane (fig. 8). The sporozoites then slowly worked their way through the gap, leaving an empty husk containing several refractile granules (fig. 12).

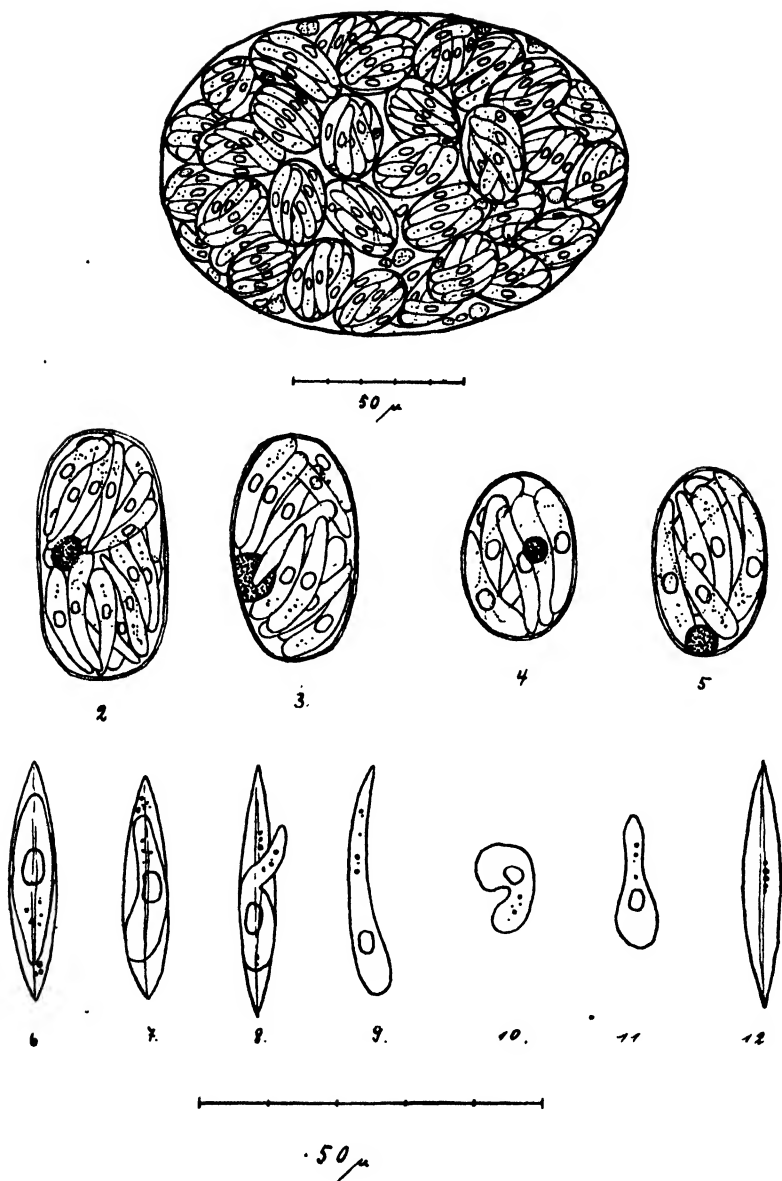
Having escaped, the sporozoites continue their contractile movements, constantly changing their shape and, at the same time, performing a slow translatory movement.

All the material was transferred to two slides and examined in the fresh.

As it seemed obvious that the oocyst above described formed a part of the life-cycle of a haemogregarine of a vertebrate, the following experiments were immediately performed.

(i) The contents of one fresh preparation containing intact sporocysts and numerous free sporozoites from ruptured sporocysts were carefully washed off the slide in 0.5 c.c. normal saline ; 0.25 c.c. of the resulting mixture was injected intraperitoneally into a specimen of *Gongylus ocellatus*, and the remainder intraperitoneally into a specimen of *Mabuia quinquefasciata*. The above two lizards were both free from haemogregarines at the time of the experiment. The gecko *Hemidactylus turcicus*, which is common in houses in Jericho and feeds on sandflies, would have been a more suitable animal for the experiment, but unfortunately no specimen of this animal was at the moment available in the laboratory.

(ii) The material from the second fresh preparation containing numerous sporocysts and free sporozoites was rubbed into puncture



FIGS. 1-12.

1. A complete oocyst.
- 2-5. Sporocysts.
- 6-7. Sporozoites in their enclosing membrane.
8. Sporozoite escaping from membrane.
- 9-11. Change in shape of a sporozoite.
12. Empty membrane with refractile granules.

wounds made by a needle into the forearms of two healthy human beings.

The lizards were examined at intervals during an observation period of five weeks and the peripheral blood was found to contain no haemogregarines. At the end of this period the two animals were killed and the liver, lungs and bone marrow were examined for schizonts with a negative result. The blood of the two human beings was also found to be negative during this period.

The oocyst described above apparently belongs to the genus *Hepatozoon* (Miller 1908) since it contains numerous sporocysts and each sporocyst contains a number of sporozoites, the formula for the genus *Hepatozoon* being, according to Reichenow (1921) :

'Oocysts with n sporocysts, sporocysts with n sporozoites.'

Infection of a new vertebrate host with *Hepatozoon* sp. takes place by the accidental ingestion of the transmitting arthropod containing ripe oocysts, e.g., *Mus rattus* becomes infected with *Hepatozoon perniciosum* (Miller 1908) by swallowing the mite *Lelaps echidninus* containing ripe oocysts, and dogs are infected with *Hepatozoon canis* (James 1905) by swallowing infected *Rhipicephalus sanguineus*.

Up to the present the genus *Hepatozoon* has been recorded only from mammals, but the finding of oocysts belonging to the genus *Hepatozoon* free in the abdominal cavity of *Gl. palpalis* by Chatton and Roubaud (1913) and by Macfie (1916) points to the possible presence of this genus in lizards or birds which feed on the fly.

In the case of the oocyst of *Phlebotomus papatasi*, two possible modes of infecting a vertebrate host suggest themselves :

1. Ingestion by a lizard and liberation of the sporozoites into the alimentary canal.
2. The crushing of an infected *Phlebotomus* during the act of feeding, and the liberation of sporozoites into the wound, i.e., the method by which *Phlebotomus* is generally assumed to transmit cutaneous leishmaniasis. The experiment on two human beings described above thus approximates to an infection under natural conditions.

A number of observers (Krempf 1917, Dimond 1917, Sergeant, Et. and Ed. and Parrot 1922, Noc 1922, Nattan-Larrier 1922), have described haemogregarines from man. The findings of all these authors have been subjected to a destructive criticism by

Wenyon (1923), who concluded that 'the haemogregarines of man have still to be found.'

The bionomics of *P. papatasii* render it an eminently suitable transmitting agent of a haemogregarine to man if such occur and the result of the experiment on two human beings is therefore of interest.

The above is the first record of an oocyst in *Phlebotomus papatasii*.

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THE GENUS *TETRACAMPOS* WEDL, 1861

BY
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Woodland, in the '*Annals of Tropical Medicine and Parasitology*,' Vol. XIX, No. 2, p. 185, refers the above genus to the *Bothriocephalidae*.

In a former issue of the '*Annals*,' I gave certain reasons for referring the genus to the Order *Cyclophyllidea*.

This difference of opinion cannot, unfortunately, be settled by an examination of the worm in question, viz., *T. ciliotheca* Wedl, 1861, because the material is not available.

As the species is stated by Wedl to possess four lappets or bothridia which are figured, it should, on that account, be referred to that Order of Cestodes which is characterised by the possession of four bothridia, viz., the *Tetraphyllidea*. Owing to the fact that Wedl's figure of the head leaves one in considerable doubt as to whether the so-called bothridia are really bothridia, or whether, on the other hand, they are badly figured acetabula; and also having in mind the fact that other cestode parasites with armed heads bearing true acetabula, and with ventral pores, have been repeatedly obtained from fish closely related to that in which *T. ciliotheca* was found, the writer concluded that Wedl's genus *Tetracampos* belonged to the *Proteocephalidae*; and, as in this family the head is armed with four suckers, it was referred to the Order *Cyclophyllidea*.

Up to the present helminthologists have agreed, and rightly, that the primary divisions of the polyzootic cestodes should be made on the character of the head. Thus, in the *Cyclophyllidea* the head bears four suckers, in the *Tetraphyllidea* four bothridia or lappets, in the *Trypanorhyncha* four proboscides, and in the *Pseudophyllidea* sometimes one or more, but usually two, bothria (or grooves).

The head thus provides a ready and eminently satisfactory means of effecting a natural classification of this group of worms into

Orders, and the utility and simplicity of this means of classification justifies us in retaining it, until a better system is provided.

In the absence of a head, it is frequently impossible to refer a cestode worm to the Order to which it belongs. If the genital pores (excluding the uterine pore or pores, whether primary or secondary) are situated on the ventral surface, the worm is placed in the Order *Pseudophyllidea*; there are, however, exceptions to this rule.

If the genital pores are lateral, then it is necessary to locate the position of the vitelline glands. If this organ consists of numerous follicles situated laterally, it is still impossible to say whether the worm belongs to the Order *Tetraphyllidea* or to the Order *Trypanorhyncha*.

If the gland is single, the worm is referred to the Order *Cyclophyllidea*. Unfortunately, however, there are a number of species which, although they possess a head typical of the *Cyclophyllidea*, have the vitelline glands arranged along the lateral margins, and there are also a few species which, while characterised by having a *Tetraphyllidean* head, have the vitelline glands condensed into a single mass situated behind the ovary.

The male and female genital organs are of the same type, especially in species of all the three Orders, *Cyclophyllidea*, *Tetraphyllidea* and *Trypanorhyncha*, the trivial differences which exist being limited to the disposition of the musculature, the number of testes, the size of the cirrus pouch, the position of the pore on the lateral margin, etc.—points obviously only of importance in the differentiation of species, or at most of genera. The form of the uterus in the *Pseudophyllidea* is, however, usually characteristic in that Order. .

In spite of the fact that in *T. ciliotheca* the head bears four bothridia, or four suckers, Woodland refers the genus to the Order *Pseudophyllidea*, and states that 'scolex characters count for very little.'

Woodland realises that the head of a *Bothriocephalid* usually possesses two bothria, for he states that the four bothridia in *T. ciliotheca* 'are evidently the four walls bordering the bothriae or sucking grooves.' For a similar reason one could consider the Order *Tetraphyllidea* identical with the *Pseudophyllidea*.

It is true that Wedl states that in *T. ciliotheca* the embryophore is ciliated exactly as it is in *D. latus*. Practically nothing is known

regarding the eggs of the *Tetraphyllidea*, and for this reason one cannot say whether the fact that the embryophore in *T. ciliotheca* is ciliated, has any particular significance or not.

Woodland states that other typically Bothriocephalid features of *T. ciliotheca* are: (1) the shape of the anterior proglottides; it is not stated what this character is, and the writer's experience is that the anterior segments are almost always featureless; and (2) the ventral position of the genital apertures. It has already been pointed out that the uterus in many species of *Proteocephalidae* bursts to the exterior by a slit or a number of slits on the ventral surface, and it is not impossible that what Wedl called a genital pore was a uterine opening.

Referring to the *Proteocephalidae*, Woodland further writes 'for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the *Tetraphyllidea*.' It is common knowledge amongst all who have worked with worms of this order, that although in gravid segments the uterus sometimes bursts to the exterior by a slit or slits situated on the ventral surface, the presence of true uterine pores has only been established in about six species. Further, the vitelline glands are not in every case situated laterally.

Woodland's paper is useful in that his figures help one to realise pointedly the wide difference between the head of *T. ciliotheca* and those of the two other species which he considers so closely allied to it.

A NEW MEDIUM FOR THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

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During the course of an investigation into the bacteriology of the water supplies of the colony the repeated isolation of *B. coli* (lactose +, indol +) and of Houston's atypical *B. coli* from small quantities (1 c.c., .1 c.c., and .01 c.c.) of raw natural waters which were declared on sanitary survey to be free from all possibility of human faecal pollution and very remotely, if at all, liable to any animal contamination, centred attention on the sufficiency of the methods for the bacterioscopic examination of waters recommended by a Committee of the Royal Institute of Public Health in 1903, and adopted by bacteriologists in England and elsewhere as the standard method, and the interpretation of the results obtained by this method.

The literature of the bacteriology of tropical waters reveals frequent references to the presence of *B. coli* as detected by the standard method in waters which would be certified as pure and free from objectionable pollution by the comparative freedom from water-borne diseases among those drinking such waters, and by the results of sanitary surveys.

Daniels (1908), working on the waters from jungle streams of the Federated Malay States, observed that it was exceptional to find a jungle stream from which *B. coli* could not be isolated in 2 c.c., even though such waters would appear to be free from human and animal faecal pollution. Archibald (1910), commenting on the waters of the Soudan, remarked :

' If samples of water taken from shallow wells or rivers in the Tropics are subjected to a few simple tests for the presence of faecal contamination, the results will often show such a state of things that no analyst in England would ever consider the passing of such waters as fit for human consumption, and yet the water from those sources is used daily by both Europeans and natives alike without any ill effects to health as far as can be told. The question naturally arises whether in the face of these existing conditions one would be justified in using European standards of water purity as a guide or whether some modification of the European standard could be generally employed in tropical climes ? '

Wise and Minett (1912) isolated *B. coli* in from 1 to .001 c.c. of raw waters from various sources used for drinking purposes by the inhabitants of British Guiana. Clemesha in India (1908-1912), struck by the high degree of 'faecal' (?) pollution in the drinking water supplies of the Madras Presidency as evidenced by the presence of *B. coli* isolated by the standard method, conducted a series of interesting experiments demonstrating the value of sunlight in the process of purification of the waters of India.

B. coli as defined by the standard method, and by Houston, embraces more than ten varieties of organisms, and though one or more varieties may be present in a water supply, the natural purification which that water undergoes from exposure to sunlight destroys those organisms which are objectionable or are evidence of objectionable pollution.

Hence the mere statement by a water analyst of the isolation of *B. coli* in a certain quantity of water is, as regards the Tropics, at all events, of comparatively little value from the sanitary point of view unless the effects of self-purification are taken into account. If, he says, the standard method be applied in its entirety to India, nearly every drop of drinking water in that country would be condemned.

In England the raw waters of the Thames investigated by Houston are waters to which sewage and other pollution gain constant access and *B. coli* in such waters would almost invariably be of faecal origin. Unfortunately the literature available locally does not record the result of quantitative examinations for the presence of *B. coli* in the raw natural waters of England, waters obtained from uninhabited regions and not exposed to human or animal faecal pollution.

Thresh, however, refers to a public water supply from the Welsh moorlands in which no sewage contamination was possible, but in which *B. coli* (lactose +, indol +) was present in 1 c.c. Such a water, he recommends, should be filtered before being delivered to the consumer. Does this example, though solitary, indicate that *B. coli* may perhaps be present in raw natural waters not exposed to human or animal pollution in temperate regions as is the case in the Tropics?

The standard method postulates that all *B. coli* are of faecal

origin without regard to the fact that such organisms are frequently found not only in small quantities of raw natural waters free from all faecal pollution, but also in *unpolluted soil*. From the soil free from faecal contamination these organisms may gain entrance to water supplies, and such supplies may be condemned by the standard method as polluted and unfit for human consumption. Chen and Rettger (1919) found 156 out of 467 (33·4 per cent.) coli-like organisms isolated from unpolluted soil to be lactose +, indol + organisms and Max Levine 37·3 per cent. out of 177 lactose fermenters of the soil to be also indol producers.

In Trinidad, of 120 cultures isolated from unpolluted soil by the standard method 42 per cent. were typical *B. coli* (lactose +, indol +) and if Houston's atypical *B. coli* be included, the percentage of *B. coli* regarded as an index of faecal pollution would be considerably higher.

If lactose +, indol + *B. coli* may be isolated by the standard method from soils to which no faecal matter has gained entrance, is a method which does not attempt to differentiate between faecal and non-faecal *B. coli* sufficient to justify an analyst in expressing an opinion upon the sanitary quality of a water, particularly if the sanitary control of that water is liable to variation? At all events, should the water analyst not attempt to indicate the faecal or non-faecal origin of *B. coli* isolated from water?

Keyes, Rogers, Clarke and others (1909-1914) showed by accurate determination of the gas volumes and gas ratios produced in the anaerobic fermentations of glucose that the non-spore-bearing lactose fermenters of faeces can be divided into two groups, one a low ratio or *B. coli* group in which the proportion of CO₂ to H₂ is almost constantly equal to 1·06 and the other a high ratio or *B. aerogenes-cloacae* group which produces considerably more CO₂ than H₂, with a wide range of ratio between these gases.

Clark and Lubs (1915) showed that in a carefully adjusted sugar medium the low ratio organisms produce a relatively high hydrogen ion concentration which can be recognised by an indicator, such as methyl red becoming red, whilst the high ratio organisms produce a low hydrogen ion concentration and methyl red becomes yellow.

In human faeces, according to Rogers, Clarke and Lubs, the low ratio group (methyl red +, Voges-Proskauer -) constitutes

74 per cent., and the high ratio group 26 per cent., of the lactose fermenters, whilst in bovine faeces the low ratio group constitutes 99·4 per cent., and the high ratio group ·6 per cent. (Rogers).

Chen and Rettger, in 1919, found all of 173 organisms from faeces to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 740 cultures isolated from human, bovine, and equine faeces, 94 per cent. were methyl red positive and all Voges-Proskauer negative.

On the other hand, Chen and Rettger found that of 467 coli-like organisms isolated from unpolluted soils, 430 belonged to the high ratio (methyl red —, V.-P. +) or *B. aerogenes-cloacae* group, and 20 to the low ratio *B. coli* group and, as stated above, 33·4 per cent. of these 467 were lactose +, indol +.

In Trinidad, of 120 cultures isolated from unpolluted soil, 85 per cent. were methyl red negative and 15 per cent. methyl red positive, and as previously pointed out 42 per cent. were lactose +, indol +.

But the gas ratio determination is not possible in the ordinary laboratory analysis of water, and whilst the methyl red and Voges-Proskauer tests have been found in the case of faeces and soil to indicate fairly accurately the habitat of the organism under investigation, their application in the bacteriological analysis of waters has been shown to afford no clue as to the source (faeces or soil) of the organism isolated from a water. Thus Winslow and Cohen found the percentage of methyl red positive, Voges-Proskauer negative organisms to be practically the same in polluted, unpolluted and stored raw waters. Out of 255 coli-like organisms, 76 per cent. from unpolluted, 77 per cent. from polluted and 85 per cent. from stored rain water were methyl red positive and V.-P. negative. Stewart Koser found 80·4 per cent. of the colon group cultures obtained from polluted waters and 73·3 per cent. from unpolluted waters to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 220 organisms isolated from polluted waters, 87·3 per cent. were methyl red positive, 6·3 per cent. methyl red negative, and 6·4 per cent. doubtful; and of 240 cultures obtained from sanitarily pure waters 42·5 per cent. were methyl red positive and 57·5 per cent. negative. Though the American Public Health Association (1923 ed.) recommends the methyl red and Voges-Proskauer tests in the bacteriological examination of water, local

experience supports the conclusion of Winslow, Cohen, Stewart Koser and others that the lack of correlation between these tests and the sanitary qualities of waters justifies little reliance being placed upon them as indices of sanitary purity.

Stewart Koser (1923), in a study of the utilisation of salts of various organic acids, found that the two sections of the colon group of organisms could be clearly distinguished by the use of a chemically definite medium containing sodium, potassium or ammonium citrate as the only source of carbon. Such a synthetic medium can be made by dissolving 1.5 grammes microcosmic salt $\text{Na}(\text{NH}_4)\text{PO}_4 + \text{H}_2\text{O}$, 1 gramme KH_2PO_4 , 0.2 gramme MgSO_4 and 2 grammes sodium citrate in 1000 c.c. distilled water, tubing, and autoclaving at 120°C . for fifteen minutes. A clear colourless liquid is obtained. In this medium Stewart Koser found that 90.7 per cent. of *B. coli* isolated from faeces failed to develop, whilst the *B. aerogenes-cloacae* group produced a visible turbidity within forty-eight hours at 30°C . This differentiation correlated with the methyl red and Voges-Proskauer tests as far as the typical *B. coli* type and the aerogenes section in faeces are concerned.

With regard, however, to organisms isolated from the soil, he found that a number were consistently methyl red positive and Voges-Proskauer negative, although they had been obtained from soils regarded as free from pollution. When tested in the citrate medium these soil coli were found to utilise it. Of 72 cultures obtained from unpolluted soils, 97.2 per cent. utilised the citrate with the production of a visible turbidity and were distinct from faecal *B. coli*, whilst the methyl red test showed 51.4 per cent. alkaline to methyl red and the Voges-Proskauer 52.8 per cent. positive.

In Trinidad, of 432 cultures isolated from human, bovine, and equine faeces, 96.3 per cent. failed to develop in the citrate medium, while 3.7 per cent. did so; and in the case of unpolluted soils, of 214 cultures of the coli group, 90 per cent. utilised the citrate medium in forty-eight hours and 10 per cent. failed to do so. The citrate medium as a biological test is thus an accurate indicator of the habitat of the coli organism isolated from faeces and soil. In the application of the citrate medium for the differentiation of faecal and non-faecal *B. coli* obtained from waters, very striking results have been obtained. Samples of waters were secured from localities

in Trinidad where the chances of human intestinal pollution were impossible and contamination by birds and an occasional wild animal practically negligible ; by the standard method, *B. coli* were isolated and put through the citrate, methyl red and Voges-Proskauer tests. Of 240 cultures thus obtained, 81.3 per cent. grew in the citrate medium and 18.7 per cent. failed to do so; whilst of 210 *B. coli* isolated at various periods from polluted streams below villages 90.9 per cent. failed to utilise the citrate and 9.1 per cent. produced a distinct turbidity. The citrate utilisation by *B. coli* is thus seen to afford some degree of correlation with the sanitary survey of a water supply. Further, the *B. coli* colonies (lactose +, indol +) isolated from a certain quantity of water, say 1 c.c., by the standard method, may, by the citrate test, be shown to be of non-faecal origin and it is only in a larger quantity of water (5.10 or 25 c.c.) that faecal (citrate -) *B. coli* (lactose +, indol +) is found. Whilst, therefore, by the routine standard method a water may be condemned, by the use of the citrate test the bacteriological analysis of a water supply may be found to harmonise with epidemiological and sanitary conditions. To those, therefore, engaged in water analysis in the Tropics, particularly of those waters where that perfect sanitary control obtained by Sir Alexander Houston for the waters of the Metropolitan Water Board can only be an impossible vision, Stewart Koser's remarks should be of special interest. He says :

' the primary results shewn by the citrate medium indicate that this method of differentiation is deserving of further study with regard to its usefulness and application in the sanitary examination of water supplies, though the final acceptance of any such test must of course await general confirmation at the hands of different workers.'

Such a test is necessary. For is the relatively low incidence, in certain parts of the Tropics, of water-borne diseases, in contrast with the high degree of faecal pollution as evidenced by the presence of *B. coli*, detected by the standard method, due to the constant accidental absence of the specific pathogenic organisms or to the natural purification which waters in the Tropics undergo from exposure to sunlight in addition to the fact that by the standard method no attempt is made to differentiate between faecal and non-faecal *B. coli*?

SUMMARY

1. *B. coli* (lactose +, indol +) may be isolated by the standard method not only from faeces and polluted waters, but also from unpolluted soils and unpolluted waters.

2. As a standard indicator of faecal contamination its value is not therefore unquestionable.

3. Local experience indicates that the utilisation of citrate by *B. coli* may be of value in differentiating faecal from non-faecal *B. coli* in water analysis.

UNPOLLUTED SOIL

| | IN TRINIDAD | | IN AMERICA (Chen and Rettger) | | IN AMERICA (Levine) | |
|--|--------------|-------------------------------|----------------------------------|-------------------------------|------------------------|-------------------------------|
| | | No. of Colonies studied | | No. of Colonies studied | | No. of Colonies studied |
| Lactose + Indol + <i>B. coli</i> | 42 per cent. | 214 | 33.4 per cent. | 467 | 37.3 per cent. | 177 |

CITRATE TEST IN AMERICA (STEWART KOSER)

| | Growth in Citrate | No growth in Citrate | No. of Colonies studied |
|---------------------|-------------------|----------------------|-------------------------|
| Faeces... .. | 9.3 per cent. | 90.7 per cent. | 118 |
| Unpolluted Soil ... | 97.2 per cent. | 2.8 per cent. | 72 |

CITRATE TEST IN TRINIDAD

| | Growth in Citrate | No growth in Citrate | No. of Colonies studied |
|----------------------|-------------------|----------------------|-------------------------|
| Faeces... .. | 3.7 per cent | 96.3 per cent. | 432 |
| Polluted Water ... | 9.1 per cent. | 90.9 per cent. | 210 |
| Unpolluted Soil ... | 90 per cent. | 10 per cent. | 214 |
| Unpolluted Water ... | 81.3 per cent. | 18.7 per cent. | 240 |

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MALARIA INFECTION AS IT OCCURS IN LATE PREGNANCY ; ITS RELATIONSHIP TO LABOUR AND EARLY INFANCY

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PLATE VI

I. INTRODUCTION

In a previous paper (1925) we published an account of the malaria incidence in a series of twenty-six placentas of native women in Freetown. The investigation of placental malaria has been continued on all material available since then, so as to eliminate seasonable variations, and the records now cover a period of a complete year, i.e., from July, 1924, to July, 1925. Examinations have been made not only of films of the placental blood and of the peripheral blood of the mother at and about the time of labour, but also of cord and peripheral blood films of the children born of mothers with infected placentas. A small amount of material has been obtained from post mortem examination of children born dead or who died within a period of seven days. For purposes of comparison certain figures of the infection rate of the adult male population have been introduced.

The distribution of parasites in infected placentas has been studied with a view to discovering whether the whole placenta is equally infected or whether there is special concentration of the parasites in any particular areas.

Evidence of the transmission of parasites from the placenta to the child has again been sought. Although evidence of such transmission of parasites has never once been obtained throughout the whole series, yet there are certain facts which strongly suggest that the presence of malaria in the placenta is frequently associated with abnormal labour, that the death-rate among children born of mothers with infected placentas is unusually high, and that the

blood of the child is deleteriously affected by the parasitic invasion of the placenta. Little material from cases of abortion was available and what was obtained was not usually suitable for examination. It is not possible, therefore, to adduce any facts to show whether malaria is an important factor in the causation of abortion in Freetown or not. This is an aspect of the case which clearly requires attention, but until greater facilities for obtaining material are made it is not possible to advance much in this direction.

In our previous paper, we discussed the view held by some observers that new-born children of infected mothers possess a temporary and partial tolerance as regards malaria, so that a new-born child, although congenitally infected, does not present parasites in the peripheral blood at birth nor until after the lapse of a certain time. We argued that there was no direct evidence of the existence of such a partial tolerance on the part of the child, and that there was evidence against its existence at least in some cases, i.e., where authentic congenital malaria has been demonstrated. We noted, however, that of 41 children of one month or under, only one, a child between three and four weeks old, had parasites in the peripheral blood. In this present series it will again be seen that children under one month rarely show parasites in the peripheral circulation. This freedom from parasites in the peripheral blood may be due to freedom of the child from infection. If this is so, it may merely result from the fact that, for some unexplained reason, children up to a week or two old are little exposed to the bites of infected anophelines. On the other hand, it would be compatible with either a temporary general immunity, i.e., a condition during the existence of which the child is totally incapable of developing infection anywhere, or a condition of local immunity with partial tolerance, i.e., a condition in which the child is in fact infected, but in which the infection does not appear equally distributed throughout the body, but only in certain parts, of which the peripheral circulation is not one. That such local infections do occur in adult women, and, moreover, that they can very frequently exist without the production of obvious constitutional symptoms, we are able to prove conclusively from the present series.

We shall show that of 150 parturient native women, aged 15 to 42, examined during the period of twelve months, 55 proved to be

infected with *P. falciparum*. Of those infected, however, only 10 showed infection of both peripheral and placenta blood. In the remaining 45 only the placenta was infected.

It must be concluded, therefore, either that in these latter cases the parasites remain localised in the placenta, and never leave it, or else that if they do leave the placenta on their way to the peripheral blood via the vena cava, they are rapidly destroyed; for it seems impossible to explain merely on the ground of dilution, the non-appearance of parasites in films from the peripheral circulation, when we consider that the placenta is a highly vascular organ, that it represents some $\frac{1}{16}$ th of the total body weight and that of the maternal erythrocytes which it contains as many as 65 per cent. may be infected, as was shown by us previously (1925). See Plate VI, fig. 1.

It seems not only legitimate but necessary to believe that in pregnant native women infected with malaria, there are certain portions of the circulatory system which are immune from infection; while at the same time, in the same individuals, other portions, far from being immune, exhibit massive infection, accompanied by active sporulation. If we admit a local immunity in the case of the mother, it must be admitted that a similar condition may exist in the child. Although in no case in a child born of a mother with placenta infected were parasites found either in the cord, peripheral blood, or in such organ smears as were available, we are not in a position to deny the possibility of malaria parasites establishing themselves in the internal organs of the child although not appearing in its peripheral blood. We can say, however, that such a condition, while it would be in accordance with the idea of the existence of local immunity in one portion of the child's circulation, namely, the peripheral blood, would equally imply the absence of such an immunity in another portion, namely, the umbilical cord. This question will be referred to again in discussing the age incidence of malaria infection in the children, and the fact that in a few cases of children born dead or who died immediately after birth, there was found in smears made from the internal organs, pigment which could not be distinguished from malaria pigment.

Before proceeding to a detailed account of the facts obtained, it may be noted as somewhat extraordinary that since the observa-

tions made on placental infection by the Greek observers Pezopoulos and Cardamatis (1907), little attention has been given to the discrepancy which appears to exist between the infection rate of males and females as judged by the peripheral blood rate of the former and the placental blood rate of the latter; nor, in our opinion, has sufficient attention been attached to this method of diagnosing malaria in the case of parturient women whose peripheral blood has yielded no evidence of it.

II. EXAMINATION OF THE PERIPHERAL BLOOD

A. *Of mothers.*

Thin film preparations of blood were made from the peripheral blood of 173 mothers at the time of labour; in addition to thin films, thick films were also examined in 71 of these cases. The number of cases in which malarial infection was diagnosed by examination of the peripheral blood was 12, of which 9 had parasites of *P. falciparum*, and 3 pigmented leucocytes only; it is noteworthy that in no case were gametes found, although in 5 of the 9 positive parasitic cases, one or more thick films were also examined.

Seasonal incidence of infection in the peripheral blood of mothers.

The number of mothers examined by films of the peripheral blood varied from 5 to 24 in a month; the total number examined and the approximate percentage found positive in each month are shown in Table I.

TABLE I.

Showing monthly total of mothers examined and percentage positive.

| | Total | Percentage positive | | Total | Percentage positive |
|------------------|-------|---------------------|-----------------|-------|---------------------|
| July | 8 | 25 | January | 17 | 6 |
| August | 5 | 20 | February | 5 | 0 |
| September | 10 | 10 | March | 7 | 0 |
| October | 16 | 0 | April | 27 | 7 |
| November... .. | 24 | 8 | May | 25 | 4 |
| December | 13 | 8 | June | 16 | 13 |

B. *Of non-parturient women.*

Of 43 women of the age of 16 and upwards, all, however, examined by means of thick film preparations from the peripheral blood, three had parasites. One of these was a *Plasmodium vivax* infection, the other two were *P. falciparum* infections, and in one of the latter crescents were present.

C. *Of Adult males.*

In a series of 150 males of the age of 16 and upwards, all examined by the thick film method, three had trophozoites of *P. falciparum* in the peripheral blood ; in no case were crescents found.

D. *Of children.*

Each one of a series of 809 children of all ages up to two years and a half was examined, on its first appearance, by thin film preparations of the peripheral blood ; an additional examination was made at the same time in the case of 100 of these children by the thick film method. Of the 809 children, 169, i.e., 20.9 per cent. had parasites in the peripheral blood ; *P. falciparum* occurred alone in 149 cases, *P. malariae* alone in 12 cases, and *P. vivax* alone in 2 cases ; mixed infection of *P. falciparum* and *P. vivax* occurred in 2 cases, of *P. falciparum* and *P. malariae* in 2 cases, and of *P. falciparum*, *P. malariae*, and *P. vivax* in 1 case. One case diagnosed by pigment alone cannot be classified. *P. falciparum* infection was found therefore in 19.0 per cent. of the 809 cases examined. Crescents were present in 23 cases, i.e., 14.9 per cent. of the 154 *P. falciparum* cases ; this percentage is low, as in cases in which trophozoites were found at once, the examination was not continued to the time limit pre-arranged.

Seasonal incidence of malaria in the peripheral blood of 809 children up to 2½ years.

The monthly total number of new children examined by films of the peripheral blood varied from 36 to 104 ; the total examined and the percentage found positive in each month are shown in Table II.

TABLE II.

Showing monthly total of new cases examined and percentage positive.

| Month ... | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | April | May | June |
|-----------------------|------|------|-------|------|------|------|------|------|------|-------|------|------|
| Total ... | 62 | 78 | 93 | 60 | 36 | 51 | 77 | 57 | 104 | 40 | 87 | 64 |
| Per Cent. positive... | 24.1 | 24.4 | 26.8 | 21.7 | 30.6 | 25.5 | 22.1 | 28.1 | 13.5 | 10.0 | 17.2 | 18.7 |

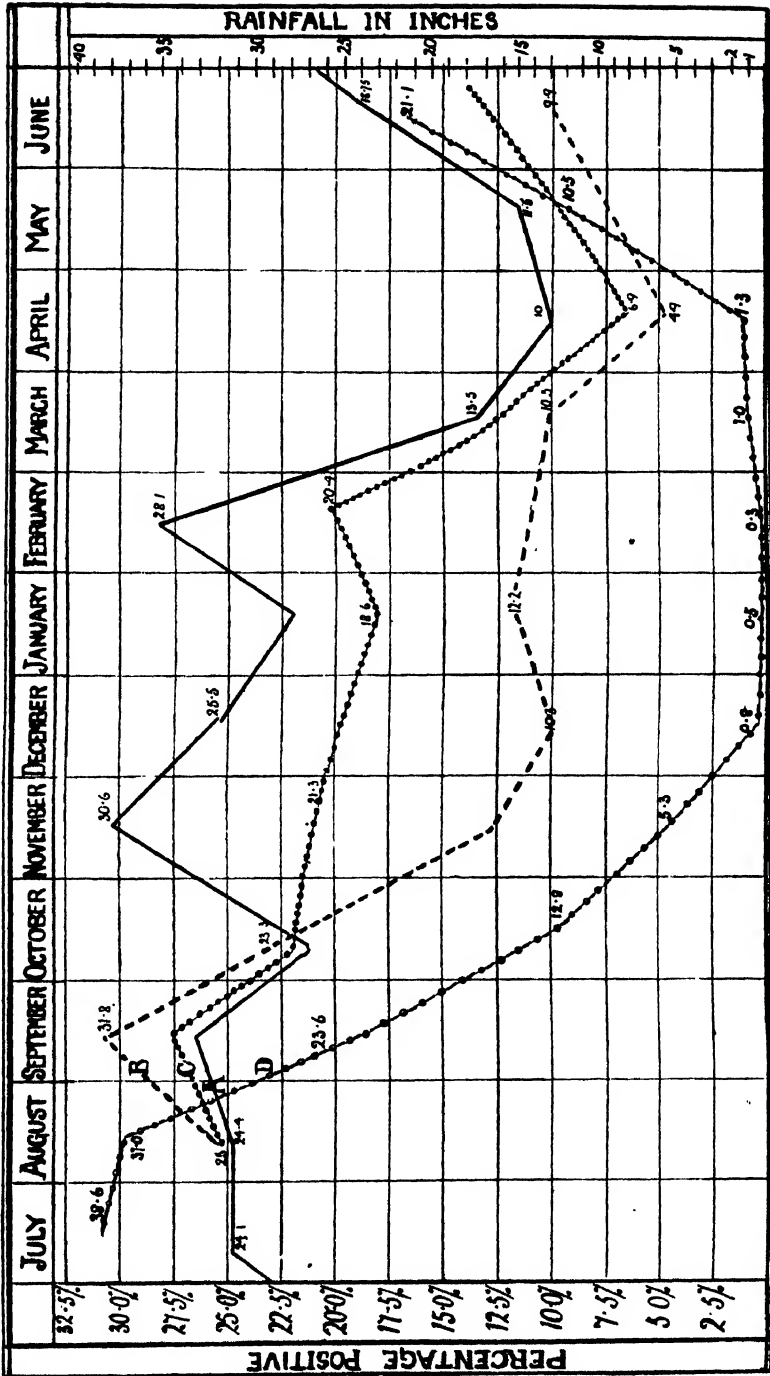
Each case on its first appearance is classified here as new ; on any subsequent appearance, therefore, it is classified as old, and the parasitic findings require separate record, as is shewn in the graph given below.

The number found positive among new cases in each month is expressed in Graph I A as a percentage of the total new cases appearing for examination for that month. The number of old cases found positive for the first time in each month is expressed in Graph I B as a percentage of the corrected total of old cases seen in that month. The corrected total of old cases is arrived at by excluding all cases which had previously been found positive. The numbers found positive in the above two categories are added together and expressed in Graph I C as a percentage of the total cases, i.e., new cases plus corrected old cases, appearing for examination in each month. The rainfall in inches in an average year is shown in Graph I D.

Table II and Graph I A, which give the same information, represent the results of a single examination ; Graph I B represents the results of at least two, and it may be numerous examinations and includes all cases which, negative on first examination, proved positive at a later time ; the resultant curve of the summation of the positives in A and B shown in Graph I C gives a more accurate impression of the seasonal incidence of malaria in the children than does A alone or B alone.

It is seen that the form of curve c which we regard as yielding the most reliable information on the question of seasonal incidence in children, presents a fairly definite relationship to the rainfall curve. The relationship is such as to show that soon after the commencement of the rains the malaria incidence rises. If we compare the peripheral

GRAPH I.
Showing seasonal incidence of Malaria and average rainfall in inches.



blood of children with the placentas of mothers (Section IV, Table V) we see that in the latter a rise occurs antecedent to the rains and before the rise in the children's infection. This suggests that the placental rise represents a seasonal relapse, while that of the children represents a seasonal infection.

III. EXAMINATION OF BLOOD FROM THE UMBILICAL CORD

Thin films of blood from a vessel in the umbilical cord were examined in 162 umbilical cords. In no cord was malaria infection found, in spite of the fact mentioned below that the examination of 155 of the placentas belonging to the cords revealed 59, i.e., 38 per cent. positive; further, in spite of careful search, no pigmented leucocyte was ever found in the cord blood.

Maternal leucocytes in the foetal circulation.

It is stated* that leucocytes can penetrate from the maternal into the foetal circulation; this statement is based upon the relative numbers of leucocytes found in the umbilical arteries and vein. It has been found that the umbilical vein blood contains per volume a greater number of leucocytes than the umbilical arterial blood. This passage of leucocytes presumably occurs through the walls of the villi and is a matter which requires further investigation where placental infection with malaria occurs. It may not be justifiable to argue that where leucocytes can pass through, erythrocytes can also pass through; still less is it justifiable to assume that infected erythrocytes can pass; for it was observed by Marchiafava and Bignami (1894) that in capillary apoplexies almost all the extravasated red blood-corpuscles were without parasites, while the cerebral vessels contained immense numbers of red blood-corpuscles having parasites in them. It is, however, important to note that in these heavily infected placentas it is by no means rare to find leucocytes which contain within them parasites. These parasites may be of any size up to forms which are segmented and appear ready to rupture. It is not possible to say whether leucocytes containing such parasites are capable of penetrating from the maternal to the foetal circulation, nor is it possible to say whether such parasites, if liberated in the child's circulation, could infect it. All that can be said is that certain of these parasites contained in leucocytes were undoubtedly alive at the time of examination as evidenced by their movements visible

by the dark ground illumination method. These parasites containing leucocytes are, comparatively speaking, rare, and it is unlikely that even if they penetrated into the child's circulation, they would come under observation. In a number of placentas examined the majority of the leucocytes contained pigment (see Plate VI, fig. 2).

If the difference noted by some observers between the total leucocytes present in the umbilical cord vein and arteries is, as stated by Gray (1923), due to the penetration of the maternal leucocytes into the foetal circulation, it is difficult to account for the fact that in spite of the most careful examination we failed to find such pigmented leucocytes in either the cord or the peripheral blood of the child.

In the endeavour to ascertain whether the condition of the arterial and venous cord blood, as regards the proportion of leucocytes present, is as stated, we have in one case made careful enumeration of the leucocytes present in films of each. The result showed that such difference as existed between the leucocyte content of the venous and the arterial cord blood was negligible, but showed a slight preponderance of leucocytes in the arterial blood, in the proportion of arterial 187 and venous 170 to 50,000 erythrocytes.

IV. EXAMINATION OF BLOOD FROM THE PLACENTA

Of 155 placentas of native women examined for malaria 59 were found to be positive, that is 38.0 per cent.

The results of the different blood examinations obtained so far are set out in tabular form arranged according to the ascending percentage of parasitic positives.

TABLE III.

Showing the parasitic findings of various groups.

| | Blood films of | 162 Umbilical cords | 150 Adult males (peripheral blood) | 43 Non- parturient women (peripheral blood) | 173 Mothers (peripheral blood) | 809 Children (peripheral blood) | 150 Mothers' placental blood | 155 Placentas |
|----------------------|-------------------|---------------------------|--|--|---|--|---------------------------------------|------------------|
| Percentage having | Parasites ... | 0.0 | 2.0 | 7 | 6.9 | 20.9 | 36.6 | 38.0 |
| | Crescents ... | 0.0 | 0.0 | 2.3 | 0.0 | 2.8 | 0.0 | 0.0 |

It is interesting to compare the above placental figures with those given in Table IV taken from Clark (1915), which shows the distribution of placental infection among different races in his series of 400 labours.

TABLE IV.
400 Routine cases of labour.

| Race of the women examined | No. examined of each race | No. of positive identifications of malaria | Per cent. of positive cases |
|----------------------------------|---------------------------|--|-----------------------------|
| North Americans (white) | 118 | 0 | 0.0 |
| Latin Americans (mestizo) | 92 | 3 | 3.26 + |
| Europeans (white) | 17 | 1 | 5.88 + |
| West Indian Negroes | 173 | 15 | 8.67 + |
| Total | 400 | 19 | 4.75 |

It is seen that Clark's percentage of positive mothers is low as compared with ours, 4.75 per cent. as compared with 36.6 per cent. A partial explanation of this fact is obtainable from consideration of the groups forming his total. Thus the 118 North Americans (white) give a percentage infected figure of 0.0, whereas the 173 West Indian negroes give the highest figure a percentage infected of 8.67. The difference of incidence is attributed by Clark to the higher hygienic plane of the North Americans, to the greater exposure to infection of the West Indian Negroes on account of their residential surroundings and their much lower hygienic and economic standard. Even taking the figure for West Indian negroes alone, however, the infection rate, i.e., 8.67 per cent. does not approach that seen in West African native women in Freetown, i.e., 36.6 per cent. If placental findings are taken as a criterion of malarial infection, it appears that the West African native women in Freetown are more than four times as frequently infected as the West Indian negroes dealt with by Clark.

Seasonal incidence of infection of the placenta.

The number of mothers whose placenta was examined by blood films varied from 6 to 24 monthly during the year. The total

number examined and the percentage found positive in each month are shown in Table V.

TABLE V.

Showing monthly total of mothers examined and the percentage found positive.

| | Total | Percentage positive | | Total | Percentage positive |
|------------------|-------|---------------------|-----------------|-------|---------------------|
| July... .. | 8 | 62 | January | 18 | 39 |
| August | 6 | 33 | February | 7 | 43 |
| September | 12 | 42 | March | 11 | 18 |
| October | 16 | 19 | April | 11 | 18 |
| November | 24 | 38 | May | 15 | 53 |
| December | 13 | 31 | June | 9 | 56 |

Type of infection in the placental blood.

Without exception all the cases found positive in the placenta were infected with *P. falciparum*; in one case a few parasites which resembled quartan were also found. In spite of prolonged examination of the placental films, no crescents were ever found in the placental blood. In writing the account of our first series of twenty-six placentas we drew attention to the absence of crescents in the placental blood, and also in the peripheral blood of such cases as showed infection there. We have obtained no evidence from this larger series that crescents are being formed elsewhere in these infected individuals, as no crescents have been found in any of them in the peripheral blood during, or immediately after, labour. If post-mortem material had been available it might have been possible to determine whether any crescents were present in internal organs. Blacklock (1921) produced evidence from a case of indigenous infection with *P. falciparum* in England that the bone marrow was the most suitable site for the development of crescents, a site which had previously been stated to be favourable by Marchiafava and Bignami and various other observers.

These cases, then, although the infection in the placenta is often very intense, are not producing crescents in this site. Nor does it appear probable that the parasites are migrating from the placenta

to develop elsewhere into crescents, because we do not find crescents in the peripheral blood. The failure of sexual forms to reach the peripheral blood must inevitably result in failure of the parasite to complete its development even when susceptible anophelines bite such women. The rare possibility of parasites of the schizogony cycle being transmitted congenitally to the child must be taken into consideration ; this would doubtless result in the formation later of gametes in the child, and so in a circuitous manner the stages infective for the mosquito would become available. In the meantime we have the fact, proved by abundant evidence, that the mere proliferation of *P. falciparum* on a colossal scale in one organ at least of native adult women does not result in the production of crescents in that organ, nor does it result in their appearance in the peripheral blood.

The anatomy and circulation of the placenta.

Before discussing the distribution of malaria parasites in the placenta, it is necessary to make some reference to the placental circulation. According to the descriptions and diagrammatic representations of the placenta and its circulation contained in many text books of anatomy, the condition is somewhat as shown in the left hand side of diagram No. 1. The arteries and vein of the child are carried from the umbilical cord, and pass subjacent to the amnion into the chorion ; the vessels are carried into the villous processes of the chorion ; the villi lie in the intervillous space and are bathed in maternal blood. As, however, the villi are covered by two layers of trophoblast, the cytotrophoblast layer next the chorionic process and the syncytiotrophoblast layer in contact with the maternal blood, the latter does not come directly in contact with the foetal blood vessels.

The maternal blood gains access to and leaves the intervillous space by arteries and veins which pass through the stratum spongiosum and the basal plate which represents the remains of the stratum compactum. The arteries as they enter the basal plate lose their muscular coat and they and the veins after this point consist of sinuous channels lined only by endothelium ; these channels open into the intervillous space, and at this point they lose their endothelial covering. The intervillous space is lined throughout by the syncytiotrophoblast layer. Therefore the walls of the

intervillous space and the villi which project into it are covered by the same lining structure.

The right-hand half of the figure represents the separated placenta ; according to Gray and others this separation occurs through the stratum spongiosum. On the right half of the diagram have been shown the areas in which infected and uninfected red blood

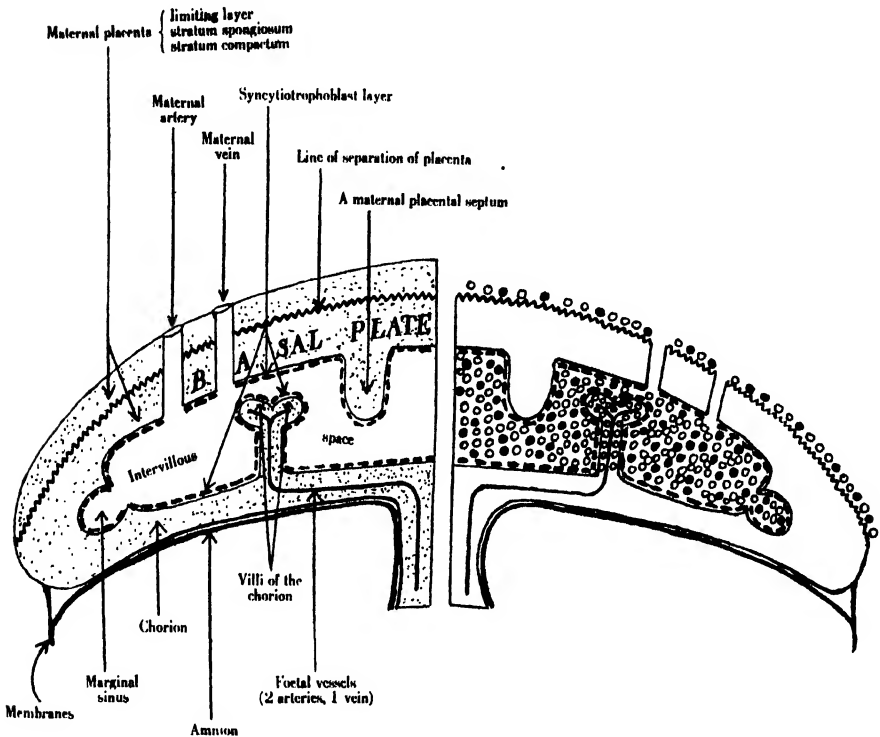


FIG. 1. Diagram of placental circulation (modified from Gray's anatomy). The left-hand figure represents the placenta before birth and the right-hand figure the same after birth. In the right-hand figure infected red cells are marked ● and uninfected red cells marked ○.

cells were found. The methods used were applied not only to the central portions of the placenta but also to the margin, and were :—

(1) The maternal surface of the placenta was carefully washed with normal saline solution ; smears were made from the surface, and also from blood obtained by scraping very lightly with a razor blade ; finally the thinnest possible slice of tissue was taken from the surface and from this thin slice films were spread.

(2) Films of blood from the placenta were examined at various depths from the maternal towards the foetal surface.

(3) The amnion was carefully washed with the normal saline solution and then reflected; from the surface thus exposed smears were made and then, as in the case of the maternal surface, a thin portion of the tissue was snipped off and smears made from this thin portion.

(4) Wedge-shaped portions of tissue from the margin of the placenta and cylindrical portions from the centre were taken in such a way as to include both the maternal and foetal surfaces and the intervening tissue; these were embedded and sectioned.

The examination showed that parasites were present in every preparation so made from infected placentas; some variation occurred as shown in the attached table, in the proportion of infected erythrocytes present in blood films made at different parts of the placenta, from the tissue snipped from the maternal and foetal surface, and from the placental tissue at a point midway between these surfaces; the results of such examinations are given in Table VI.

TABLE VI.

Showing percentage of erythrocytes infected at different parts and depths of the malarial placenta.

| Blood films from | Edge of placenta | Centre of placenta |
|---|------------------|--------------------|
| Tissue snipped from maternal surface ... | 11.0 | 18.2 |
| Tissue midway between maternal and foetal surfaces | 9.8 | 36.2 |
| Tissue snipped from foetal surface subjacent to amnion | 8.2 | 9.4 |

As is seen in the table the centre of the placenta has a larger proportion of erythrocytes infected than the edge of the placenta; further, the portion situated midway between the maternal and foetal surfaces in the central portion is the most heavily infected of all.

We were unable to account for this unequal distribution in any way except on the assumption that infected cells tend to accumulate here while uninfected cells pass on. If this area presented a more suitable medium in which the parasites could complete sporulation,

we might expect to find a greater proportion of sporulating forms of parasite in the infected cells of this area. We enumerated the sporulating forms found in each area with the result that in all areas the percentage of sporulating forms was found to be approximately the same ; for example, in one case where 200 parasites were counted in each area by each observer, the percentage of sporulating forms in each area was approximately 14.

Consideration of the distribution of parasites in relation to the anatomy of the placenta.

A point which early attracted our attention was that although the peripheral blood of the mother was free from parasites not only at the time of labour, but also in individual cases which were followed for a month after labour, yet parasites could be found on the maternal surface of the placenta in large numbers. In sections of the placenta thin walled sinuses are found near the maternal surface, some of which, presumably maternal arteries, are free from parasites, while others, presumably maternal veins, contain numerous parasites. Assuming that the condition found after the placenta is delivered were in existence before separation of the placenta, it is difficult to avoid the conclusion that parasites must be present in the maternal veins of the placenta in large numbers. Possibly this is so, and the failure to find these parasites in the peripheral blood may be due solely to the peripheral immunity which we have already postulated.

We believe, however, that the distribution of the parasites on the maternal surface of the placenta found after delivery may not represent the distribution as it occurs in the placenta before separation. The placenta is comparable to a flat sponge on one surface of which is the relatively thick covering membrane composed of chorion internally, and amnion externally, while on the other is the much thinner membrane composed of the remains of the stratum spongiosum externally and the basal plate internally ; internal to these is the lining of trophoblast. The intervillous space with the villi projecting into it occupies the whole area between the maternal and foetal internal surfaces.

The intervillous space everywhere extends up to the maternal surface, as well as to the foetal surface, at the margin as well as in the centre of the placenta. Consequently infected erythrocytes

contained in the intervillous space lie right against the maternal and foetal lining of syncytiotrophoblast. The processes given off into the space from the foetal surface, namely the chorionic villi, have a counterpart in the more scanty septal processes into the space from the maternal basal plate. All these processes are covered

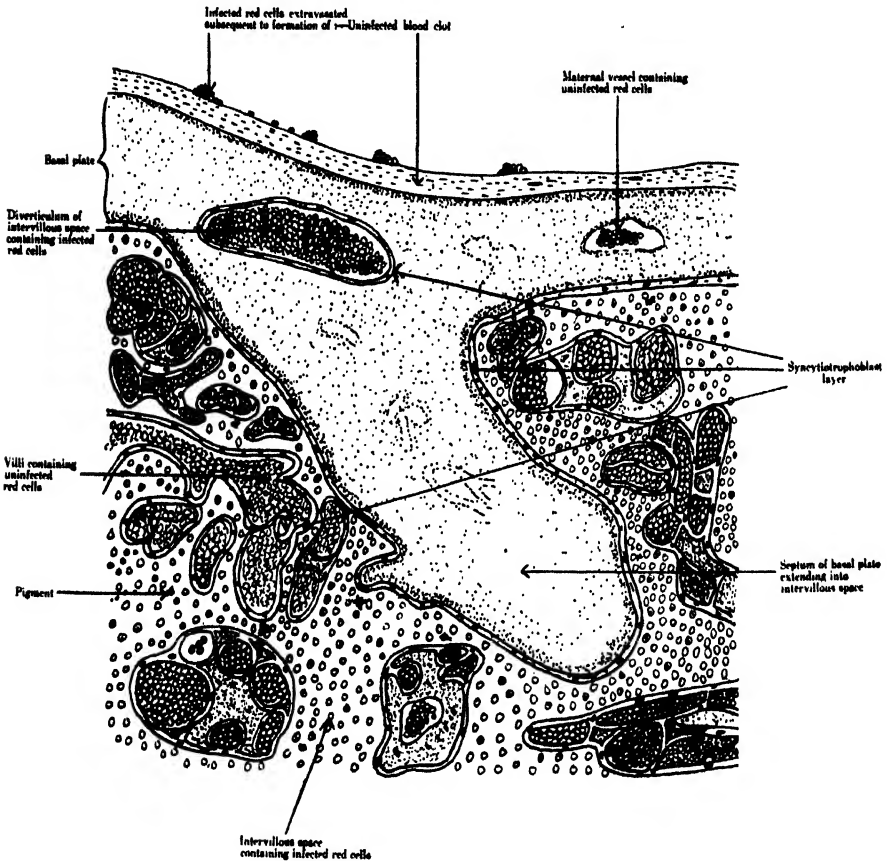


FIG. 2. Section through maternal surface of placenta [semidiagrammatic].
(Zeiss, Oc. 4. Obj. $\frac{1}{4}$ -inch.)

by syncytiotrophoblast and all are bathed in infected blood of the mother. At the origin of these processes are seen what at first appear to be areas of infected cells included in the chorion and basal plate. These are diverticula of the intervillous space cut across and are seen not only to contain infected erythrocytes

but also to be lined by syncytiotrophoblast and in some cases to contain a portion of uninfected villous process.

This arrangement in itself would suffice to explain the fact that films made from even the thinnest slice of tissue from the maternal surface or from the foetal surface after reflection of the amnion contain numerous parasites. The presence of infected erythrocytes on the uncut maternal surface of the placenta as seen after its delivery is probably brought about therefore by the aid of two factors, both of which are dependent upon mechanical compression by the uterus upon the placenta after expulsion of the child and separation of the placenta has begun. If the cord has been tied on the maternal side the uterus is contracting upon a mass of tense villi, out of which the blood cannot be expressed through the cord. As the uterine contractions continue and increase, and as the vessels of the villi remain rigidly distended with blood, diminution in volume of the placenta takes place in two ways following the line of least resistance. In the first place the maternal blood lying in the intervillous space is forced back through the remnants of the maternal arteries and veins and emerges on the maternal surface of the placenta. On further pressure the intervillous space ruptures where the membrane is thinnest, that is, through the diverticula on the maternal side, and liberates on to the surface its contained parasites.

We are inclined to believe that in normal circumstances parasites do not extend beyond the limits of the syncytiotrophoblast layer which lines the intervillous space and which invests all processes into it, whether villi of the chorion or septa from the basal plate. Parasites, indeed, as we have shown, may always be found close to the maternal and foetal surfaces and often penetrate into both, but in the latter case they are normally lying in sinuous prolongations of the intervillous space and are still contained within the limiting syncytiotrophoblast layer. We have noted that parasites have never been seen by us in the villi themselves.

Rupture of the diverticula of the intervillous space appears to be attributable primarily to ligation of the cord on the placental side. If the placental side of the cord were not tied there might still be backward oozing from the maternal vessels, but it is clear that this leakage and the leakage consequent upon rupture of the intervillous space diverticula are very much accentuated by the fact that ligation

of the cord keeps the villi which comprise a large proportion of the total volume of the placenta not only engorged with blood but practically incompressible. In some cases it is possible to see in section that the villi themselves are ruptured although this is relatively rare;*

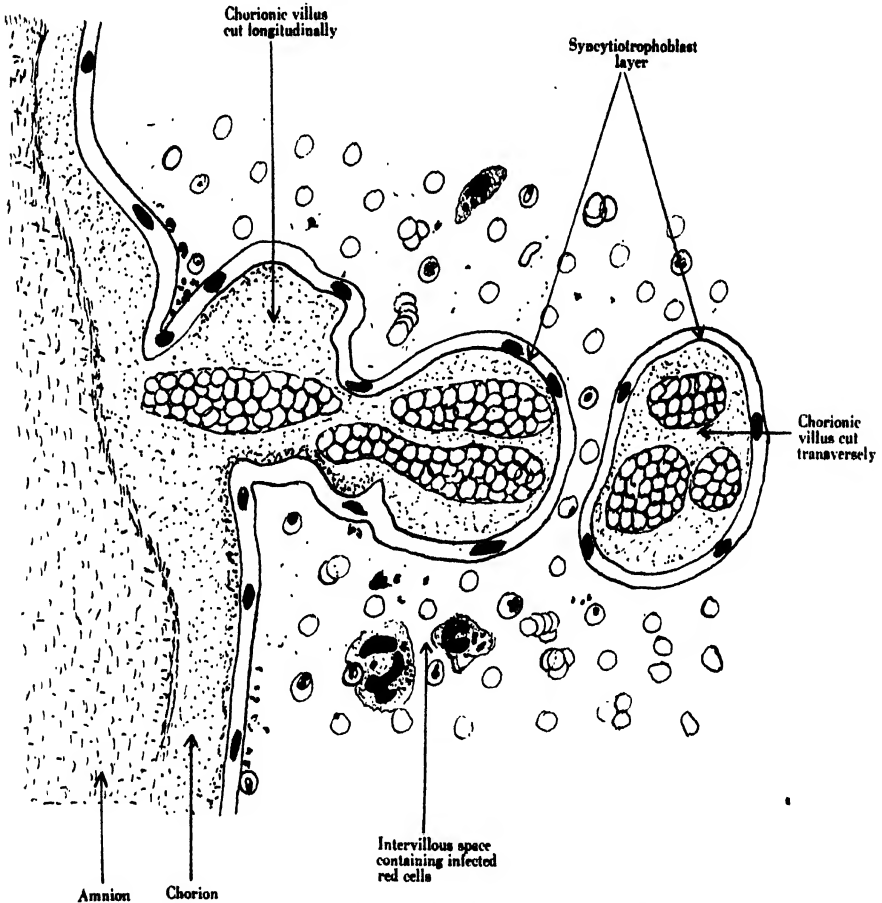


FIG. 3. Section through foetal surface of placenta, chorion and chorionic villi covered by syncytiotrophoblast layer [semidiagrammatic]. (Leitz. Oc. o. Obj. $\frac{1}{2}$. Draw tube down.)

separation and rupture of the syncytiotrophoblast layer over the villus has been observed occasionally and, still more rarely, escape of uninfected red blood cells from the villus.

* Since the above was written, one of us (R.M.G.), has had the opportunity of comparing the sections of malaria-infected placentas with sections of a large number of normal placentas at the Coombe Hospital, Dublin; this comparison showed clearly that the vessels in the villi of infected placentas are greatly dilated, in some cases to such an extent that the chorion, which normally forms the main bulk of the villus, is almost obliterated. Presumably such villi are more liable to rupture. This condition is well shown in several of the villi in Fig. 2.

If the cord were not tied it is conceivable that such rupture of villi might result in infection of the child via the cord vein ; but seeing that rupture is such a rare occurrence even when the cord is tied on the placental side, that is to say, when we have shown the conditions are most favourable to injury to the villi, it seems highly improbable that rupture of the villi could occur unless the cord is tied.

If the cord is tied on the child's side and then cut, leaving the maternal end free to bleed, it is extremely unlikely that any villi could be ruptured. In any case even if they did rupture the infection in these circumstances would obviously not affect the child via the cord. Even when the end of the cord is tied the risk of rupture of villi is extremely small, and here again the child is not exposed to risk via the cord.

There is one aspect of the question, however, which, although it is in the nature of a side issue from our investigation, appears to deserve mention. The facts here brought out have a distinct application to a controversy which has frequently arisen as to the merits or demerits of tying the placental end of the cord. The most recent exponent of the view that such ligation of the cord is injurious, is Vaughan (1925). The procedure is condemned on the ground that ligation of the cord places the uterus at a disadvantage in its efforts to contract.

We have had in this series of malaria infected placentas a unique opportunity of studying this question, and have had a method of distinguishing sharply between the foetal and maternal bloods from the circumstance that the maternal blood contained such a high proportion of infected erythrocytes in many of the cases. The information obtained by us by means of the study of malaria placentas in which the cord has been tied on the placental side enables us to bring forward new evidence to support that school which claims that the uterus when contracting on villi with cord tied is contracting on a mass which for all practical purposes is incompressible. We are, however, far from saying that we believe that this is injurious to the uterus, or that it has any deleterious effect upon its power of expelling the placenta, or upon its own final involution. These matters are outside the scope of this investigation, but we believe that what we have described may stimulate their study on the part of those whose special province it is to deal with pregnancy and the puerperium.

V. CRESCENTS

It has long been recognised that crescents appear relatively rarely in the peripheral blood of adult natives in endemic areas. Recently, Christophers (1924) has confirmed Schüffner's view that crescent formation is not associated with immunization, but that on the contrary, crescent formation is reduced during the process. The negative findings as regards crescents in the peripheral blood and placentas in this series would be in accordance with the idea that the majority of these cases were so far immunized as to prevent the appearance of parasites in the peripheral blood, and that all were so far immunized as to prevent the appearance of crescents either in the peripheral blood or in the placenta. It is of interest in this connection to note that Horowitz-Wlassowa (1924), while noting a specific antibody present in most cases of malaria, has failed to demonstrate its presence in those cases which show many schizonts or gametocytes in the cutaneous blood.

In writing of the peripheral blood of children in endemic areas, Christophers says 'Crescents here are therefore associated with the higher values of parasites, and hence one may judge with the period of acute infestation rather than with that of immune infestation.'

The absence of crescents in the placentas of the native women discussed is clearly not due to lack of parasite proliferation; it may be that the placenta is in all cases a site in which for some reason crescent formation does not occur; as suggested in our previous paper, this might be due to some intrinsic and yet unknown character of this organ which renders it unfavourable to the development of mature sexual forms. On the other hand, it is possible that the failure of crescents to develop in the placenta is an indication of a certain degree of immunity having been reached. If this is so, it would indicate that we have in the placental absence of crescents an early sign of the development of immunity. We are then faced with a complex arrangement; the patient is infected with *P. falciparum*; there exists a degree of immunity which prevents the development of crescents in the placenta as shown by direct examination of this organ, and probably in other organs as shown by the failure to find crescents in the peripheral blood; this anti-crescent immunity, then, would appear to be of a general nature affecting the whole circulatory system. Quite a different picture is presented when we examine the immunity against asexual parasites. While the

peripheral circulation appears to be immune with regard to them the placental circulation, far from being immune, offers a most suitable soil for their development on an immense scale.

It is important, first of all, to decide whether the absence of crescent formation which has throughout our series characterised the placental blood is due to an inherent character of all placentas. This could be done by examining the placentas of women who, although infected with malaria, have not resided sufficiently long in an endemic area to acquire any degree of immunity. This investigation could be carried out readily in Europe in the case of pregnant women who return after a short first residence in an endemic area, and who are infected with malaria. If it should prove that such women readily develop crescents in the placenta, it would be necessary to examine also natives of endemic areas who had gone to Europe, and who had infection of the placenta. If the latter cases still showed no crescent formation in the placenta, it would be reasonable to draw two conclusions, firstly, that the absence of crescent formation in the placenta in this series was not due to an inherent character of the placenta, and secondly, that it was due to an acquired immunity. The value of such an examination lies in the fact that if it can be shown that acquired immunity is the cause of crescent non-production, we have in the placenta an accessible internal organ which possesses very obvious advantages for the study of malaria immunity. In view of the difficulty of obtaining material here for the purpose of this study in non-immunized persons, this part of the work must be undertaken elsewhere.

It is frequently stated that crescents are produced more readily after quinine administration. Several of our series of cases had received quinine before labour, some of them for as long a period as ten days, yet in no case, as we have shown, were crescents found.

VI. PATHOLOGICAL EFFECTS OF PLACENTAL INFECTION WITH *P. FALCIPARUM* ON MOTHER OR CHILD

Before discussing any effects which might be attributed to malaria infection, we give an account of the material and examination upon which our conclusions are based. In spite of increasing efforts to obtain permission to examine material post mortem, we have still a vast amount of prejudice to overcome ; this can only be done by gradual education and awakening the interest of those most nearly

concerned, namely, the natives themselves. Our records of post mortem examinations are consequently rather meagre.

The following are the material examined and the methods adopted ; in all cases Leishman's or Giemsa's stain was used.

1. *Maternal peripheral blood.* Thin and thick films were examined from the blood of the ear, at the time of labour.

2. *Placenta.* This was usually examined within 6 hours after labour ; exceptionally as much as 24 hours elapsed before examination was possible. The surface of the placenta was washed and cauterised ; an incision was made through the cauterised area and blood from the bottom of the incision was taken up in a pipette and used for spreading films.

3. *Umbilical cord.* The cord after being washed and cauterised was cut in two places, one as near as possible to the placenta and the other about six inches from the placenta ; films were spread from the blood in the vessels.

4. *Peripheral blood of living children.* Immediately after the birth of the child, thick and thin films were made from the peripheral blood.

5. *Partial examinations of cadaver of dead born children.* In most cases it was impossible to obtain permission for a complete examination ; puncture of certain of the organs by means of a needle was permitted in some cases, in addition to examination of the peripheral blood.

6. *Complete examination of cadaver of dead born children.* Where permission could be obtained, a complete examination was made. This comprised smears of peripheral blood, liver, spleen, kidneys, bone marrow, lungs and other organs.

7. *Examination of cadavers of children who died within seven days.* The examinations carried out in these cases were of the same kind as those in number 5 and 6.

Material from post mortem examination of the children was preserved and material from the placentas was fixed, embedded in paraffin and sectioned.

A. Effect on the mother before and after labour.

In Table VII given below, the main facts concerning the 55 infected mothers and their children are set out, with special reference to the fate of the child.

TABLE VII.

Showing clinical history and peripheral blood findings in 55 mothers infected in the placenta with *P. falciparum*; also the fate of their children.

| Case No. | Age | Peripheral blood of mother | Temperature 3 days before or after delivery | Quinine, total amount given before delivery | Child born alive | Child lived 7 days |
|----------|-----|----------------------------|---|---|------------------|--------------------|
| 1 | 20 | + | 105° F. | 25 gr. | Yes | Yes |
| 3 | 24 | o | 100° F. | o | Yes | Yes |
| 4 | 21 | o | 105° F. | o | Yes | Yes |
| 5 | 35 | + | 100° F. | o | Yes | Yes |
| 6 | 36 | ... | 100° F. | o | Yes | No |
| 9 | 18 | + | N | o | Yes | Yes |
| 13 | 18 | o | 105° F. | 30 gr. | Yes | No |
| 16 | 22 | + | 103° F. | 36 gr. | Yes | Yes |
| 17 | 36 | ... | 103° F. | 5 gr. | No | ... |
| 19 | 30 | ... | ... | o | Yes | Yes |
| 20 | 26 | o | N | ... | Yes | Yes |
| 23 | 22 | o | 102° F. | o | Yes | Yes |
| 34 | 37 | o | ... | ... | Yes | Yes |
| 35 | 22 | o | ... | ... | Yes | Yes |
| 38 | 20 | o | ... | ... | Yes | Yes |
| 45 | 36 | o | N | o | Yes (2) | No (2) |
| 51 | 21 | o | 100° F. | o | Yes | Yes |

TABLE VII—Continued

| Case No. | Age | Peripheral blood of mother | Temperature 3 days before or after delivery | Quinine, total amount given before delivery | Child born alive | Child lived 7 days |
|----------|-----|----------------------------------|--|--|---------------------|-----------------------|
| 52 | 22 | o | N | ... | Yes (1) No (1) | Yes ... |
| 53 | 15 | o | N | o | Yes | Yes |
| 58 | 28 | o | 100° F. | o | Yes | No |
| 59 | 22 | + | N | o | Yes | Yes |
| 63 | 29 | + | N | o | Yes | Yes |
| 64 | 24 | o | 100° F. | o | Yes (1) Yes (1) | Yes (1) No (1) |
| 67 | 26 | o | N | o | Yes | Yes |
| 69 | 24 | o | N | o | Yes | Yes |
| 71 | 24 | + | N | o | Yes | No |
| 75 | 25 | o | N | o | Yes | Yes |
| 81 | 23 | o | 100° F. | o | Yes | Yes |
| 86 | 21 | o | N | o | Yes | Yes |
| 92 | 22 | o | ... | ... | No | ... |
| 93 | 23 | o | N | o | Yes | Yes |
| 94 | 20 | o | 100° F. | o | Yes | Yes |
| 95 | 32 | o | N | o | No | ... |
| 96 | 36 | + | ... | o | Yes | Yes |
| 98 | 20 | o | N | 50 gr. | Yes | Yes |
| 101 | 24 | ... | N | o | Yes | Yes |

TABLE VII—*Continued*

| Case No. | Age | Peripheral blood of mother | Temperature 3 days before or after delivery | Quinine, total amount given before delivery | Child born alive | Child lived 7 days |
|----------|-----|----------------------------------|--|--|---------------------|-----------------------|
| 102 | 26 | o | N | o | No | ... |
| 103 | 28 | o | N | o | Yes | Yes |
| 108 | 26 | o | 101.5° F. | o | Yes | No |
| 110 | 28 | ... | N | o | No | ... |
| 118 | 30 | ... | 102.5° F. | o | Yes | Yes |
| 128 | 28 | o | N | o | Yes (1) Yes (1) | No (1) No (1) |
| 134 | 16 | o | N | o | Yes | No |
| 136 | 17 | o | 103.8° F. | o | Yes | Yes |
| 137 | 18 | o | 101° F. | o | Yes | Yes |
| 138 | 33 | o | 101° F. | o | Yes | No |
| 140 | 26 | o | 104° F. | o | Yes (1) No (1) | No ... |
| 142 | 20 | o | N | o | Yes | Yes |
| 143 | 22 | o | N | o | Yes | Yes |
| 146 | 34 | + | 100° F. | o | Yes | Yes |
| 147 | 26 | o | N | 30 grs. | Yes | Yes |
| 150 | 24 | o | 103° F. | o | No | ... |
| 151 | 25 | o | N | 10 grs. | Yes | Yes |
| 153 | 17 | + | 102° F. | 15 grs. | Yes | Yes |
| 155 | 21 | o | ... | ... | No (1) No (1) | |

As is shown in the table 23, that is, nearly fifty per cent. of the infected mothers had fever, and three cases in which fever is not recorded received 50, 30 and 10 grains of quinine. It will be observed that in two cases in which both peripheral and placental blood was infected, namely, cases 59 and 71, there was no fever; these cases are of special interest as each presented a massive infection of the placenta. It is important to note that certain cases in which malaria was suspected to exist received quinine in varying amounts for different periods before labour without the placenta being cleared of parasites.* Conversely there are seven cases not shown in this table which were diagnosed on clinical grounds as malaria and received quinine in varying doses, and in which the placenta did not contain parasites at the time of birth. Although we know that quinine in doses quoted has failed to eradicate parasites from the placenta in the cases mentioned in the table, we cannot therefore justifiably assume that similar doses will fail in all cases. Some or all of these seven cases, had they not been treated, might have proved infected by placental examination.

In view of the number of cases who received quinine before labour and who still had infection in the placenta, it is possible that the doses were administered too late in pregnancy and in too small quantity owing to the fact that these cases only enter hospital when labour is imminent. It is known (Forchheimer (1915)), that quinine administered to the mother is excreted in the urine of the child. There were three deaths among the 150 mothers of whom the placenta was examined. None of the three who died showed malaria either in placenta nor in the peripheral blood. With regard to the post-partum history of the mothers, we have practically no information, as these cases make a very brief stay in hospital, seldom more than a week. It is therefore not possible to state whether these cases have recrudescences of malaria after leaving hospital. So far we have not had any opportunity of examining the placenta of a woman who at her previous labour had been proved to have an infected placenta.

That infected red blood cells are left behind in the uterus in large numbers when the infected placenta is born there is no doubt, for

* Since the above was written, we have had the opportunity of observing a case whose peripheral blood showed malignant tertian parasites a week before labour, and who subsequently received quinine grains 20 for six consecutive days. The placenta of this case showed a few parasites and many pigmented leucocytes.

we have already shown that parasites abound on the maternal surface of the infected placenta when born. We have also observed parasites in the blood which escapes during delivery of the infected placenta. In the process of delivery of such infected placentas this may be a source of danger where such blood is permitted to reach abrasions on the skin of the child or the attendant.

We must assume that after the expulsion of the main bulk of the parasites with the placenta, one of two events happens ; either the remaining parasites are prevented from entering the maternal circulation owing to closure of the uterine vessels, and they are then thrown out with the remains of the stratum spongiosum, or they are absorbed into the maternal circulation. In the former event the mother's peripheral and general circulation does not become infected from the placental source as a direct result of labour ; in the latter event the result would depend on two factors, i.e., the dose of parasites absorbed, and the degree of the immunity which exists in the peripheral and general circulation of the mother.

B. Effect on the children.

1. *Before birth.* A total number of 164 children were born of the 155 mothers, this figure includes premature children. The single births numbered 146, giving 146 children, and the twin births numbered 9, giving 18 children ; two was the maximum number produced at one birth. There were 148 children born alive and 16 born dead. In the case of children born alive, the only means at our disposal for ascertaining the transmission of malaria parasites or their products to the child *in utero* was the examination of the child's cord and peripheral bloods.

Of the total 148 children born alive 4 are omitted from consideration here because the placentas relating to them were not received or were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 144 there were 51, i.e., 35.4 per cent. who were born of mothers whose placenta was infected ; while 93, i.e., 64.6 per cent. were born of mothers whose placenta was not infected. Only a short examination period after birth was possible, rarely more than 7 days, but a few cases were observed for a longer period. Of the 51 children born alive, of mothers with infected placentas, 13, i.e., 25.5 per cent. died within 7 days, the

remaining 38 survived the observation period ; of the 93 children born alive of mothers whose placenta was not infected, 5, i.e., 5.4 per cent. died within 7 days, the remaining 88 survived the observation period. Of 18 children born alive, and who died within 7 days, 13, i.e., 72.2 per cent. were born of mothers whose placenta was infected, while 5, i.e., 27.8 per cent. were born of mothers whose placenta was not infected. Of the 16 born dead, 2 are omitted because the placentas relating to them were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 14 there were 10, i.e., 71.4 per cent. who were born of mothers whose placenta was infected* and 4, i.e., 28.6 per cent. who were born of mothers whose placenta was not infected.

In none of the children born alive were parasites found at birth, either in the cord or peripheral blood ; nor were pigmented leucocytes seen in any of them.

In 22 cases of dead children, i.e., some of the 10 children born dead, and some of the 18 cases who died within 7 days, we had additional means of diagnosis by post-mortem examination, a partial examination in 12 cases and a complete examination in 10 cases.

None of the 22 children who were examined by one or other of the above methods presented parasitic infection in the peripheral or cord bloods, nor in any organ examined ; nor were pigmented leucocytes found in the cord or peripheral blood of any of them. In three cases, however, pigment was found either free or contained in leucocytes in the internal organs of the child ; the mother's placenta in each of these three cases was infected with malaria. We are not in a position to state definitely what the source and nature of this pigment are ; while it may probably result from red cell destruction in the child, there is no direct evidence to show that this red cell destruction was brought about by the malaria parasite invading the red cells of the child. Our completely negative findings as regards parasites in any of the children are opposed to the idea that the pigment was produced by the parasite itself acting on the child's blood cells. We cannot exclude the possibility of infection having existed in the child and having died out before the time of examination at birth. It appears possible that toxins of malaria absorbed from

* The only case in our whole series in which infection of the placenta was diagnosed by the finding of pigmented leucocytes without parasites being present, is included in this group.

the focus of infection in the placenta will produce red cell changes in the child. In case 64 binovular twins were born with cords attached to adjacent placentas ; these placentas and cords presented remarkable differences in the blood as shown in Table VIII.

TABLE VIII

Showing the differences in the placentas, cords, and fate of the child in twins (both placentas infected).

| | Placenta | | Cord | | Child | |
|---|--|--------------------------------------|---|-------------------|----------------------|----------------|
| | <i>A</i> | <i>B</i> | <i>A</i> | <i>B</i> | <i>A</i> | <i>B</i> |
| Macroscopic appearance of placenta, and fate of child | Anaemic | Normal | Anaemic in first 6 inches only | Normal throughout | Died within 15 hours | Lived 7 days + |
| Type of parasite ... | 30 % of parasites sporulating | No sporulating form seen | ... | ... | ... | ... |
| Number of parasites | 1-15 fields | 1-25 fields | ... | ... | ... | ... |
| Microscopical appearances | Evidence of great destruction of red cells | Normal except for infected red cells | Same as placenta for first 6 inches, remainder normal | Normal | Normal | Normal |

The degree and stage of infection in the two placentas was different ; *A* having a higher degree of infection and a high proportion of sporulating forms. The uninfected red blood cells of placenta *A* presented all stages of lysis ; poikilocytosis and anisocytosis were present. Cabot's rings, pseudospirochaetes and fragmented red cells were very numerous ; on the other hand the uninfected cells of placenta *B* appeared normal.

In cord *A*, apart from the absence of parasites, the changes in the blood were identical with those seen in placenta *A* ; the changes noted were, however, confined to the first six inches of the cord nearest the placenta ; beyond this point cord *A* appeared normal ; cord *B* was normal throughout its length. The peripheral blood of both children appeared to be normal in so far as the non-nucleated red

cells were concerned, and a differential count of the leucocytes, and a comparison of the nucleated red cells showed the following results.

| Peripheral Blood Diff. Leuc. Count | P. | LM. | SM. | Eos. | Bas. | Nuc. Red. |
|------------------------------------|------|------|------|------|------|-----------|
| Twin <i>A</i> | 39.0 | 8.5 | 40.0 | 1.5 | ... | 11.0 |
| Twin <i>B</i> | 51.5 | 13.0 | 22.5 | 4.0 | 0.5 | 8.5 |

The different appearances of the blood in placentas *A* and *B* are illustrated in figures 3 and 4 in the Plate. This case is of interest in that whereas no parasites were found in the child and cord *A*, yet there was evidence in the cord blood of extensive damage to red cells similar to the damage in the placenta *A* blood.

We are compelled to leave unanswered the question what exactly is the pigment found in the organs of the child, in the cases referred to. It is suggestive, however, that in three cases in which pigment was found in the organs of the child, in each case it had died *in utero*, and that there were marked changes in the blood of the placenta belonging to each child; these changes resemble closely the appearances found in the placenta *A* and the first position of cord *A*; in case 64 no pigment was found. The remarkable appearance of the blood in a small portion of cord *A*, i.e., that nearest the placenta, and the similarity of the appearance in the blood of this part of the cord and that of the corresponding placenta suggest strongly that some agency acting in the placenta in causing destruction of red cells had also acted on the blood of the child in the portion nearest the placenta at the time the cord was tied. This agency we suggest is toxin liberated by the parasites sporulating in the placenta. If this child's cord had not been tied for some time after sporulation had occurred in the placenta, it is probable that all trace of this extensive localised destruction of red cells in the cord would have disappeared, being carried away by the circulating blood. The toxin which had in this case begun to pass into the child's blood stream was confined and prevented from circulating by the ligature of the cord, and so was acting in a concentrated form on a limited amount of blood with the results noted and illustrated. The toxic effects were

not observed in the blood of the cord at any point further away than six inches from the placenta.

In Table IX we give a summary of the salient facts concerning the children born dead or who died within 7 days, and in Table X which follows this, we give the figures which would represent the expected results in these cases if we assume that malaria had no part in the production of the mortality.

TABLE IX

Showing the number and percentage of children who were born dead or who died within 7 days, among 61 children born of 55 infected mothers and 97 children born of 95 uninfected mothers.

| | Total | Born of 55 infected mothers | | Born of 95 uninfected mothers | |
|-------------------------------------|-------|-----------------------------|------------|-------------------------------|------------|
| | | Number | Percentage | Number | Percentage |
| Children born dead | 14 | 10 | 71.4 | 4 | 28.6 |
| Children who died within 7 days ... | 18 | 13 | 72.2 | 5 | 27.8 |
| Totals | 32 | 23 | 71.9 | 9 | 28.1 |

In Table X below are given the figures which would be expected provided that malaria infection had no influence.

TABLE X

Showing the totals and percentages in each group in Table IX redistributed in proportion corresponding to the ratio of the infected to the uninfected mothers, i.e., 55 infected to 95 uninfected in a total of 150 cases.

| | Total | Born of 55 infected mothers | | Born of 95 uninfected mothers | |
|-------------------------------------|-------|-----------------------------|------------|-------------------------------|------------|
| | | Number | Percentage | Number | Percentage |
| Children born dead | 14 | 5.1 | 36.4 | 8.9 | 63.6 |
| Children who died within 7 days ... | 18 | 6.6 | 36.7 | 11.4 | 63.3 |
| Totals | 32 | 11.7 | 36.6 | 20.3 | 63.4 |

From a consideration of these two tables we can conclude, if such a small group can be taken as representative, that malaria here has a definite and important effect in the production of a high proportion of infant deaths *in utero* and in the first week of life.

It is difficult to say whether any isolated group of figures is representative of the true facts among a large population, but when it is remembered that these cases here discussed comprise the vast majority of all cases treated in the maternity hospital, and that they include members of every important tribe living in Freetown, we may legitimately assume that they form a fair sample of the urban population in this endemic area.

We believe that almost conclusive evidence is provided by the figures considered above ; but over and above this we have obtained from the study of a large number of infected and uninfected placentas data which convince us that the pathological alterations in the malaria-infected placenta are such that they cannot fail to have a deleterious effect on the child in one or more ways.

(1) *Congenital malaria.* In spite of the enormous infection seen in many placentas we have not seen any parasitic evidence of this condition. We are, therefore, in a position to repeat for this larger series of cases what we said in our previous account of our first 26 cases, namely, that this condition is of great rarity. In view of this it is interesting to note that in other countries very different results have been obtained ; for example, Ziemann (1924) records that Weselko, in 1922, in Albania attributed to congenital malaria the death in the first week of 144 children of mothers infected with *P. falciparum*, while Swellengrebel (1925) records 48 cases of congenital malaria in the near East in each of which a microscopical diagnosis was made at periods varying in time from 1 to 5 days after birth.

(2) *Interference with the nutrition of the child.* We have shown that in some cases as many as 65 per cent. of the red blood cells in the intervillous space are infected. It appears certain that in so far as the red cells are concerned in the nutrition of the child their function must be very seriously interfered with.

(3) *Toxic effects.* It is evident that large amounts of malaria toxins are being produced in heavily infected placentas. It is possible that the toxins produced by the malaria parasite are, as Ziemann (1924)

suggests, anchored in the maternal tissues, or they may be incapable of reaching the foetal circulation ; but the similarity of the blood changes observed in many infected placentas and in the placental portion of the cord in such instances as case 64 quoted above lead us to suppose that the toxins are at least in some cases capable of penetrating into the child's circulation. It appears probable from the above facts that definite effects on the child are brought about by the two last factors, that is to say, interference with nutrition and toxic absorption.

VII. THE AGE INCIDENCE OF MALARIA

(1) *Mothers.* The age incidence of 55 cases of malaria occurring among 148 mothers is shown in Table XI.

TABLE XI

Showing the distribution according to age of 148 maternity cases and of 55 placental malaria infections amongst them.

| Years | Total maternity cases | Total malaria cases | Percentage infected |
|---------------|-----------------------|---------------------|---------------------|
| 15-20 | 32 | 12 | 37.5 |
| 21-25 | 55 | 20 | 36.4 |
| 26-30 | 37 | 13 | 35.1 |
| 31-35 | 8 | 5 | 62.5 |
| 36-40 | 14 | 5 | 35.7 |
| 41-45 | 2 | 0 | ... |
| Totals | 148 | 55 | 37.2 |

Excluding the 41-45 age period in which the figures are exceptionally small, the general effect of the table is to show that parturient women at all ages are equally susceptible to malaria infection in the placenta. Taking this table in conjunction with Table VII it will be observed, in so far as clinical manifestations of malaria and effect on the children are concerned, that there is no outstanding difference between the ages groups. It is surprising to observe that some very young mothers, age-group 15-20, e.g., case 53 age 15, and case 9

aged 18 in Table VII with placenta infected can not only pass through labour without clinical manifestations of malaria infection, but can also give birth to apparently perfectly normal children. That such tolerance exists only in some cases is, however, exemplified by case 13 aged 18, and case 153 aged 17.

(2) *In adult males and adult non-parturient females.* The figures we have in these groups have already been mentioned, i.e., adult males 150 and adult non-parturient females 43. As the total infection in these groups as judged by examination of the placental blood was only 2 per cent. and 7 per cent., respectively, a curve plotted from them would yield little information as regards the age incidence.

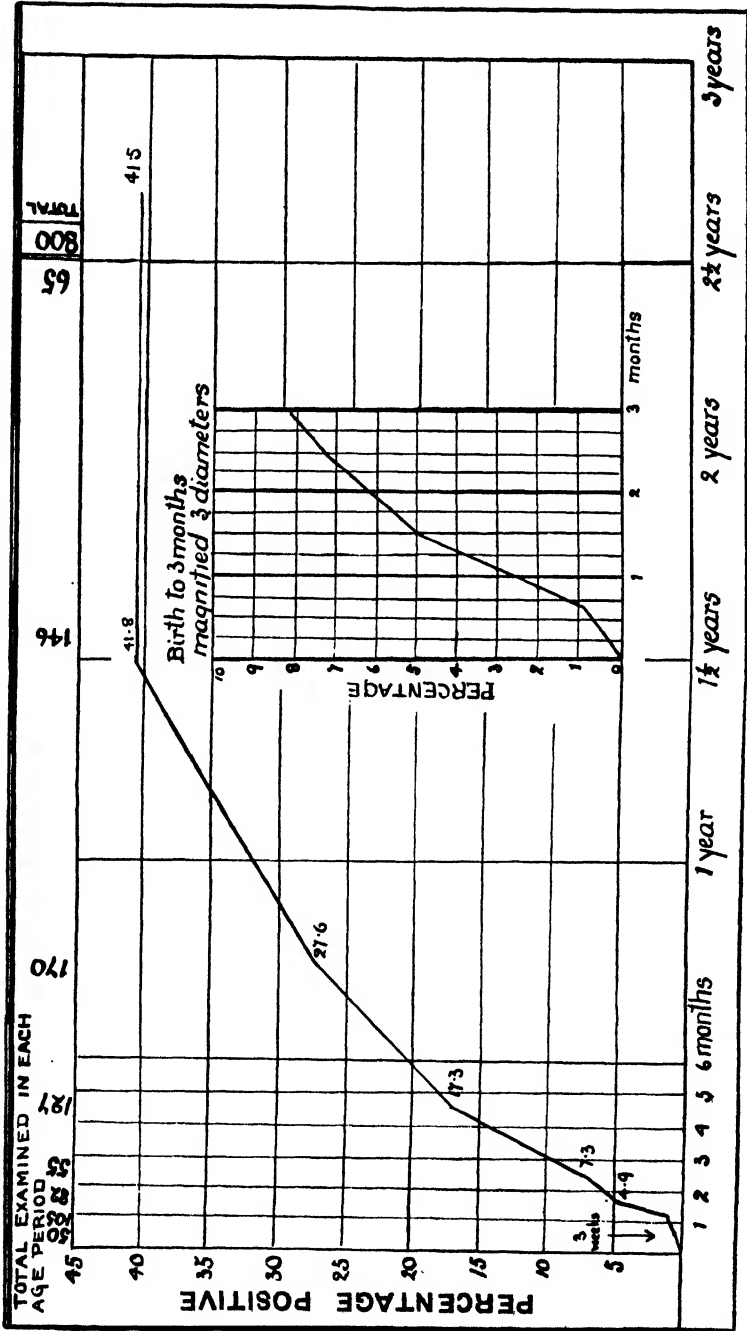
(3) *In children.* The peripheral blood of 158 children born of 150 mothers of whom 55 were infected in the placenta, and 95 were not so infected, proved negative as regards malaria at birth. In addition we have examined with negative results the peripheral blood of 41 new born children of 36 mothers; the placentas of these mothers were not examined for malaria, but in 35 of them the peripheral blood was examined with the result that two were found positive, diagnosed by the finding of pigmented leucocytes. We were unable to follow the progress of these 199 hospital cases in order to ascertain when they would become infected. The only information to be derived from them in this connection is that at birth and for a period of a week or so after birth no infection was found in any of them. From an infant clinic, however, we are able to provide the figures already dealt with in Table II from which we can show the age distribution of malaria among 800 young children, namely a series of children of ages up to $2\frac{1}{2}$ years.* In each case of this series the peripheral blood was examined once. In Graph II is shown the distribution according to age of infected cases among these 800 children examined, the examinations extending over a period of a complete year.

It is seen that only one case is recorded as positive during the first month of life. From this time on to the age of $1\frac{1}{2}$ years the infection in children shows a regular rise. The character of this curve could be explained in either of two ways. It would be

* Nine children under $2\frac{1}{2}$ years who appeared in the total for seasonal incidence cannot be included here on account of uncertainty as to their exact age.

GRAPH II

Showing distribution according to age of the malaria infected cases among 800 children at their first appearance.



compatible with the idea that the child at birth was endowed with a passive immunity derived from the mother which steadily diminished until the age of $1\frac{1}{2}$. It would also be compatible with the idea that effective exposure to infection increases steadily as the child grows older until it reaches the age of $1\frac{1}{2}$. The flattening of the curve between the ages of $1\frac{1}{2}$ and $2\frac{1}{2}$ (which is as far as our figures go) would, if the passive immunity theory is accepted, almost certainly be due to the gradual acquirement of active immunity by the child after this age, which increases directly in proportion as the passive immunity wears off. The second explanation appears less probable since it fails to explain the flattening of the curve.

CONCLUSIONS

1. Malaria infection of the placenta is very common in native women in Sierra Leone ; it occurs in about 36 per cent. of cases.

2. Infections demonstrable in the peripheral blood are in comparison few, not only in parturient women, but also in non-parturient women, adult males, and even in children.

3. The difference between the placental and peripheral incidence is in all probability due to a peripheral blood immunity.

4. These intensely infected women are practically negligible as a source of danger to the community, so far as gamete production and consequent anopheline infection are concerned.

5. Pathological effects on the mother may be entirely lacking.

6. The children may in some cases be born and survive, and may appear free from any effects.

On the other hand this series contains in other cases proof of a very complete association between maternal infection in the placenta and death of the child *in utero*, or within a 7 day observation period after birth. Our examination of a large number of infected placentas shows that severe destruction of the erythrocytes may occur in the umbilical cord in spite of the absence of congenital malaria.

The effects upon the child, noted by us, are explicable on the grounds that toxic substances are absorbed from the intensely infected placenta of the mother, and that the accumulation of masses of infected red cells interferes seriously with the nutrition of the foetus.

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ERRATA

VOL. XIX. No. 3

Page 319. For 'recommended by a Committee of the Royal Institute of Public Health in 1903' read 'recommended by a Committee of the Royal Institute of Public Health in 1914.'

Page 360. For 'examination of the placental blood' read 'examination of the peripheral blood.'

EXPLANATION OF PLATE VI

P. falciparum in the placenta.

Fig. 1 Microscopic field from an intensely infected placenta.

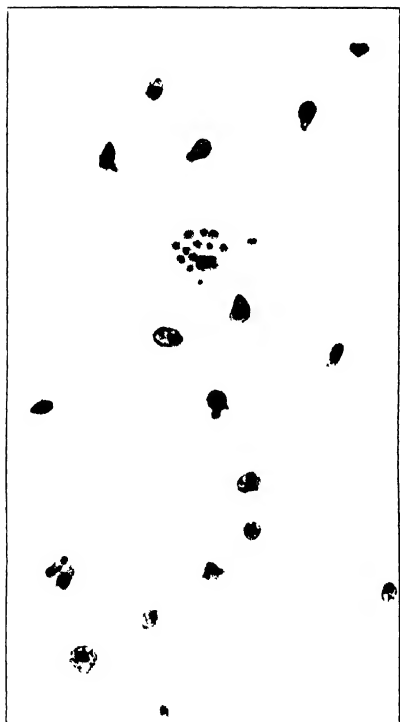
Fig. 2. Intensely pigmented type of placenta. Drawn from the tail of the film.

Fig. 3. Case 64. Placenta A.

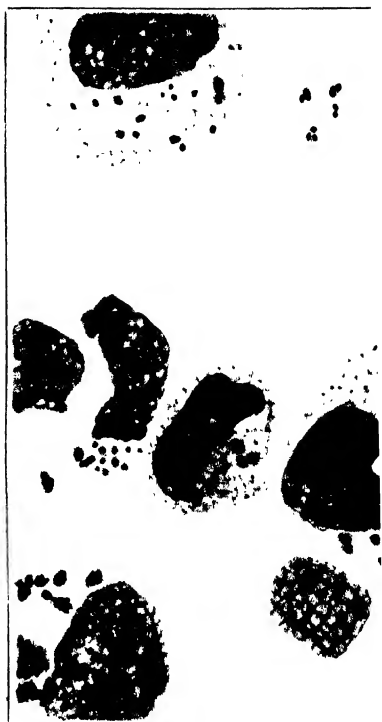
Fig. 4. Case 64. Placenta B.

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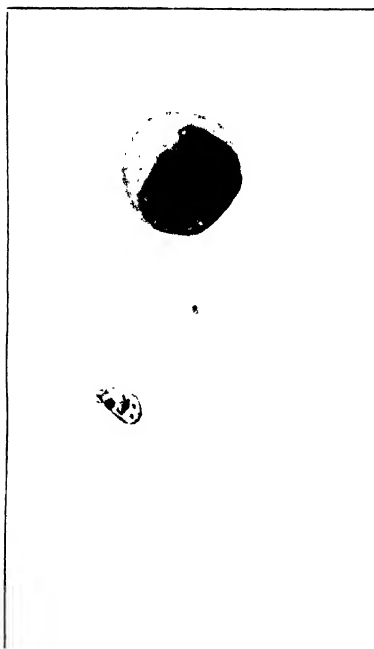
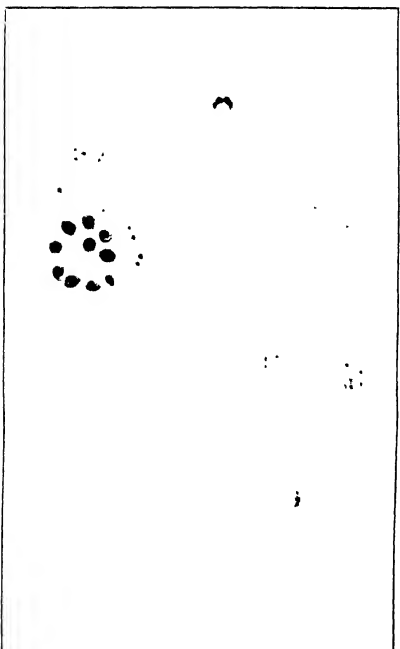
Page 342, Fig. 2. For 'through material' read
'through maternal.'



1.



2.



THE EXPERIMENTAL TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

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Sandflies have been suspected by various authors of being the transmitting agent of oriental sore. The first definite evidence in favour of the *Phlebotomus* theory of cutaneous Leishmaniasis was provided by Wenyon (1912) who found about six per cent. of the sandflies he dissected in Aleppo infected with *Herpetomonas* resembling cultural forms of Leishmania. Wenyon states that all intermediate stages of development between the small non-flagellated bodies and the fully developed flagellates occurred. Later Mackie (1914) found ten per cent. of *Phlebotomus minutus* in Assam infected with *Herpetomonas* but Patton (1922) has pointed out that oriental sore is not endemic in Assam. In Mesopotamia where oriental sore is common and sandflies are a pest, Patton (1919) states that *Herpetomonas* is present in *Phlebotomus papatasi* and *P. minutus*, and in 1922 the same author remarks on the presence of *Herpetomonas* in *Phlebotomus papatasi* and *P. minutus* in Palestine.

Additional evidence for the *Phlebotomus* theory was adduced by Acton (1919) who showed that the distribution of oriental sores on the body corresponds to the distribution of bites of *Phlebotomus*.

In Palestine the epidemiological evidence for the *Phlebotomus* theory of transmission of oriental sores (Jericho Boils) is ambiguous but rather favourable to the *Phlebotomus* theory. Canaan (1916) who first demonstrated Leishman-Donovan bodies in oriental sores from Jericho considered that town to be the only endemic centre of cutaneous Leishmaniasis in Palestine. Later however Kligler (1923) reported three cases from Kantara, Dostrowsky (1925) described ten cases from Artuf and also found one case from Bethlehem and one from Mozza, a small village near Jerusalem.

The aetiology of the disease as described by Dostrowsky in Artuf is of great interest. The population of Artuf is one hundred and fourteen and until 1923 sandflies were not observed in Artuf according to the statement of Dr. E. Jaruslawsky, the local physician (sandflies when present in numbers never pass unobserved). In the summer of 1923 the insects had for the first time become a pest to the villagers. This fact raised Dr. Dostrowsky's suspicion that the *Phlebotomus* was the carrier of oriental sores.

In June, 1924, one of us (A.) on Dr. Dostrowsky's suggestion examined the village and found every house infested with *Phlebotomus*. Three species *P. papatasii*, *P. minutus* and *P. perniciosus* were present, *P. papatasii* being by far the commonest. Dr. Dostrowsky then examined the population (ninety-seven) of an Arab village only one hundred metres from Artuf. Material was taken from every suspicious papule and given to one of us (A.). On examination Leishman-Donovan bodies were absent in every case. An examination of this village made by one of us (A.) did not reveal a single specimen of *Phlebotomus*. This may be explained by the fact that this village consists of mud huts without windows and uniformly dark in the interior, while the first village consists of fairly modern houses with whitewashed interiors, the furniture and clothes producing shade and contrast to the whitewashed walls. In Jerusalem, Jericho and Artuf it was noted that *Phlebotomus* prefers the shade produced by contrast to uniform darkness.

In Jericho *Phlebotomus papatasii* and *P. minutus* occur in large numbers, the former species predominating. *P. perniciosus* is absent or very rare. In Mozza *P. papatasii* and *P. perniciosus* are both common. It would seem then that *P. papatasii* is the carrier of cutaneous Leishmaniasis in Palestine, it being the only species common to three localities where cutaneous Leishmaniasis occurs. Schroetter, (1923), incriminated an insect which he called *Phlebotomus el Ghor* as the carrier in Jericho but Martini has shown that this insect is not a *Phlebotomus* and is incapable of biting.

Against the *Phlebotomus* theory is the fact that cutaneous Leishmaniasis is absent from many localities in Palestine where sandflies are a plague, e.g., the village of Rehoboth containing twelve hundred inhabitants where all three Palestinian species

of *Phlebotomus* abound. In Haifa (population about 30,000) which is the place most infested with sandflies in the whole of Palestine, all three Palestinian species being present, oriental sore is hitherto unknown. Still more striking is the fact that up to the present no locally acquired cases of cutaneous Leishmaniasis have been noted in Jerusalem itself. There are always a number of cases of cutaneous Leishmaniasis in Jerusalem from Jericho, Bagdad, Aleppo and Persia. In addition *Phlebotomus papatasi* is very common, *P. perniciosus* also occurs, and *P. minutus* is very rare, i.e., there are, according to the *Phlebotomus* theory, ideal conditions for the spread of the disease. Were even a small number of cases present they would not pass unnoticed for all classes of the population of Jerusalem assiduously attend the numerous clinics of the city for even the most trivial maladies and physicians are on the look-out for a case of locally acquired oriental sore.

It would seem then that on the assumption that a *Phlebotomus* sp. is the carrier of the disease in nature a third and hitherto unknown factor apart from human cases and insect carriers is necessary for the spread of the disease. What this factor is still remains to be investigated.

EXPERIMENTAL TRANSMISSION TO HUMAN BEINGS

Sergeant, Parrot, Donatien and Béguet (1921) first described the experimental transmission of oriental sore to a human being. These authors divided five hundred and fifty-nine sandflies into twenty-three batches, crushed them in saline and used the resulting material for inoculation into the arms of twenty-three volunteers. The material was collected at Biskra, an endemic centre of oriental sore, and the experiments were performed at Algiers where oriental sore is unknown.

Only one experiment from a batch of seven specimens of *Phlebotomus papatasi* gave a positive result. The experiment was performed on the 20th August, 1921; two months and twenty days later a papule was noted, and on the following day numerous Leishman-Donovan bodies were found in the papule.

In October, 1924, one of us (A.) commenced an examination of *Phlebotomus* in Jericho. Two hundred and twenty specimens of

Phlebotomus from Jericho, of which one hundred and seventy-four were females, were dissected during October to December, 1924, and three females gorged with mammalian blood were found to contain *Herpetomonas* in their midgut. All stages from non-flagellated forms to long flagellated forms were noted.

The following is a method recommended for the examination of *Phlebotomus* for *Herpetomonas*. If the insect contains no blood, cut off the terminal abdominal segment, stroke the upper surface of the abdomen with a needle to push out the ova, gently pull the head with one needle, holding the other needle against the upper surface of the thorax. In this way the head, oesophagus, salivary glands, oesophageal diverticulum and midgut are removed together, and the individual parts of the alimentary tract can be examined for *Herpetomonas*. If the midgut contains blood gently pull the head away from the thorax. The salivary glands, oesophagus, and oesophageal diverticulum and occasionally the upper part of the midgut will come away together with the head. The rest of the alimentary canal can then be pulled out from the hind end in the usual way.

In December, 1924, sandflies became very rare in Jericho and on the 20th December, 1924, a search through the whole town by a trained assistant yielded only four specimens of *P. papatasii*. Subsequent monthly examinations revealed no sandflies until April 20th, 1925. They were not found on April 4th, 1925, but on April 20th, 1925, they were numerous, *Phlebotomus papatasii* only being present. *Phlebotomus minutus* appeared towards the end of June.

A batch of one hundred and ninety-eight sandflies of which one hundred and ninety-one were *P. papatasii* (one hundred and seventy-five females and sixteen males) and seven *P. minutus* (six females and one male) were collected in Jericho on the 25th June, 1925, and brought to Jerusalem for dissection. Of this batch only one specimen, a female *P. papatasii*, was found to contain *Herpetomonas*. The insect contained no trace of blood and the abdominal cavity was full of ripe or almost ripe eggs. The whole alimentary tract was found to be swarming with *Herpetomonas*. Flagellates were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. They were especially numerous in the upper part of the midgut where swarms of parasites appeared to be attached to the posterior surface of the oesophageal valve, so much so that some parasites appeared at first sight on examination in the fresh preparation to be intracellular. (The oesophageal valve is a well-marked structure in *Phlebotomus*.)

The fact that flagellates were noted in the oesophageal diverticulum is of great interest for in freshly dissected specimens it is frequently seen that waves of peristalsis pass from the posterior end of the oesophageal diverticulum towards the oesophagus, which in *Phlebotomus* is very short, so that the oesophageal diverticulum practically opens into the pharynx. It is thus possible that flagellates may be propelled into the pharynx and buccal cavity and the possibility of a direct infection by the bite of a sandfly must be considered. Hitherto it has been generally held that infection takes place only through the crushing of an infective *Phlebotomus* on the skin, a theory which is very feasible in view of the large number of sandflies crushed on the skin (e.g. Cornwall, 1922). Another point of great interest was observed, i.e. the flagellates were not polymorphic as in the cases observed by Wenyon (1912) and by one of us (A.) in 1924, but they were all elongated and had long flagella. The oesophagus, together with the piece of oesophageal diverticulum and the upper part of the midgut, containing the oesophageal valve, were dissected away together, and another part of the midgut behind the oesophageal valve was dissected off separately; these parts were placed on separate slides, fixed in absolute alcohol, stained overnight with Giemsa, differentiated with a 0.02 per cent. solution of acetic acid and permanently mounted.

The remainder of the material was used for inoculation into the forearm of a volunteer. The volunteer had previously been exposed for three years (1917-1920) to oriental sore in Mesopotamia without contracting the disease. Two points on the skin of the left forearm were scarified and material containing flagellates was rubbed into each on the 26th of June, 1925. On the 31st July, 1925, a small papule which would normally have passed unobserved was noted on one of the inoculated points and on examination Leishman-Donovan bodies were found. The incubation period was thus less than half that noted by the Sergeants and their collaborators (1921).

The dissection of individual sandflies and experimenting with material from one infected individual is more satisfactory than crushing large numbers in saline and experimenting with the product, for in the latter case it is impossible to know whether a negative result is due to the fact that none of the sandflies contained

Herpetomonas or whether the *Herpetomonas* was non-infective. From April to June, 1925, a thousand and thirty-seven sandflies collected in Jericho were dissected and found negative for *Herpetomonas*; on the 9th of June another two hundred sandflies from Jericho were collected and on dissection found negative. Thus the experiment of crushing more than twice the number of sandflies from an endemic centre used by the Sergeants and their collaborators and inoculating a single volunteer would have given a negative result.

Nothing was noted on the other site of inoculation; nevertheless the part was scraped and on the 31st of July, 1925, examined for Leishman-Donovan bodies with a negative result. It was again examined on the 4th of August, 1925, with a negative result.

The successful results of the above experiment and the experiment of the Sergeants and Parrot, Donatien and Béguet prove that human beings can be infected with oriental sore by inoculation with *Herpetomonas* from *Phlebotomus papatasi*, but the epidemiological evidence that *P. papatasi* is the only carrier of the disease in nature is not yet complete.

In view of the successful infection with Leishmaniform parasites by injection of *Herpetomonas ctenocephali* into mice, rats, and a dog, and by injection of *Crithidia fasciculata* into mice and rats (Laveran and Franchini, 1913), by injection of *Herpetomonas jaculum* from the water scorpion *Nepa cinerea* into mice, by feeding a puppy on dog fleas (Fantham and Porter, 1915), by injecting *Herpetomonas jaculum* and *H. culicis* into birds (Fantham and Porter, 1915) the possibility of other sources of infection apart from *P. papatasi* must be considered in spite of the fact that the distribution of sandflies corresponds more closely than that of any other biting insect to the distribution of oriental sore. It must be pointed out, however, that among others Hoare (1921) working with *Crithidia melophagia*, *Herpetomonas jaculum* and *H. calliphorae* on mice, sticklebacks, newts and frogs, and Shortt (1923) working with *Herpetomonas ctenocephali* and *H. lucilae*, failed to infect monkeys, dogs, rabbits, rats, mice, pigeons and frogs.

The question of other insect carriers of oriental sore apart from *Phlebotomus papatasi* can only be cleared up in the future by direct experiments on human skin with flagellates from various insects.

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CO-ORDINATION OF EFFORT IN TSETSE-FLY INVESTIGATIONS

*(A paper read at the second Imperial Entomological Conference,
London, 15 June, 1925)*

BY

WARRINGTON YORKE

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I have been asked by the Administration of Northern Rhodesia, which I have the honour of representing at this Conference, to put before you the case for co-ordination of effort by the various African Colonies in Tsetse-fly investigation, principally in the experimental determination of the Game-fly relationship. It gives me considerable satisfaction to open a discussion on this subject, as I have long felt the necessity for co-operation of effort if any definite advance of knowledge is to be achieved. In fact, it will be within the recollection of certain here present that I pressed this point of view at the first Imperial Entomological Conference, five years ago, and at a subsequent meeting of the Royal Society of Tropical Medicine, when certain recommendations of the Glossina Sub-Committee of the Bureau of Entomology came up for review.

Now let me make it clear at once that nothing which I shall say to-day is in anyway directed against entomological research. As I said five years ago, I do not wish to place difficulties in its way, but I desire most sincerely to encourage it; and have the greatest admiration for the work which is being done by the various entomologists scattered throughout Tropical Africa, who, at considerable risk to themselves, are doing their utmost to advance knowledge. But I have long held, and I see no reason to change my views, that the problem is not a purely entomological question and that, in devising any plan of research, we must bear this in mind. The real problem is, of course, Trypanosomiasis of man and his domestic animals, and it comprises four factors: (1) the

pathogenic virus or trypanosomes; (2) the population and the domestic stock; (3) the transmitting agent or tsetse-fly, and (4) the reservoir of the virus or big game. In my judgment substantial advance in knowledge can only be achieved by research carefully devised and adequately co-ordinated with the object of taking into consideration, at the same time and in the same locality, all of the above factors. In short, I advocate centralization of effort.

What has been done in the way of research into the Trypanosomiasis problem since the Glossina Sub-Committee of the Imperial Bureau of Entomology published its report five years ago? Probably only those who, like myself, have to read and summarise the innumerable papers dealing directly and indirectly with this subject are in a position to realise the enormous amount of human energy which is being devoted to it. There are the entomological papers of Lloyd, Swynnerton, Carpenter, Fiske, Schwetz and others; the very able and suggestive epidemiological papers of Duke; the dozens of papers dealing with the action of various drugs on infected man and stock, not to mention equally numerous papers of a purely academic nature. These reports are scattered throughout a vast range of journals and periodicals. I can assure you the mere task of reading and summarising them for the *Tropical Diseases Bulletin* is no small undertaking. While the reviewer is filled with admiration for the energy and enthusiasm shown by these reports, he is only too conscious of the fact that the energy is often misdirected, that the reports, although often very long, frequently, owing to the omission of some essential information, do not permit of any inference, that even work admirably conceived and executed is often brought to nought by the fact that those who are conducting it go on leave without arrangements being made to carry it on. As an illustration of this one cannot do better than cite his own experience in attempting to summarise the position of knowledge regarding the therapeutic action of certain drugs in trypanosomiasis. Time and again one finds a record of most carefully conducted observations on long series of patients; then at periods varying from a few months to a year after the commencement of the work the observer goes on leave, or is placed on some other duty, and nothing more is heard of the patients. Now we can only judge of the result of treatment by ascertaining what has happened after the lapse of a

number of years, and as this information is in the vast majority of instances not forthcoming, the initial excellent work is thus rendered useless and knowledge or, shall I say, ignorance, remains *in statu quo*.

I had a letter the other day from Dr. Lloyd, who, as you know, has wide experience of tsetse work and is at present investigating the subject in Nigeria. He tells me that he has fenced round a small area with the object of making a game exclusion experiment. He writes :—

‘The fence was less trouble to construct than I anticipated, and is strong enough to keep out small stuff, but would not stop a roan or buffalo. In construction, when it was about three-parts done, a herd of some thirty roan got in and were there when we went to work. The labourers started to chase them and did capture a small one, but the great beasts crashed and leaped through in twenty places and the noise frightened some pig at the pool and they drove the wire out pig-shape in a dozen spots. After it was closed it took some clearing, although the area is only a half square mile. Duiker were the worst trouble as they got into the thickets and would not be flushed. However, it is clear of antelope now. The results are promising to be of interest but I fear they will not be convincing. *Tachinoides* does not seem to be affected but *morsitans* has become very scarce compared to the control. As an anomaly the infection has gone up considerably. This shows that most of, if not all, the *morsitans* in the place are emigrants from the neighbouring belts of fly. The main point of interest is the very emaciated condition of the female flies.’

Well, gentlemen, I know Dr. Lloyd intimately, and was associated with him on the Luangwa Commission. He is an extremely able and conscientious worker and, if his foreboding should unfortunately turn out to be true and the results prove unconvincing, it will, I am satisfied, not be through any fault of his, but merely of the system under which he is working. As most of you know I have always been an ardent advocate of a large and carefully controlled experiment of game destruction in a localised area and believe that from it we should obtain information of the greatest possible value. Some of you will be relieved to hear that it is not my intention to enlarge upon this much-debated subject on the present occasion. I do not like to assume the rôle of a prophet, but I am afraid that we shall not learn much from Dr. Lloyd’s experiment. He is evidently too short of funds to carry it out efficiently and on a sufficiently large scale; he is working almost single-handed and it is very doubtful to me whether, if unassisted, he will be able to make all the observations that such an experiment demands. Finally, in order to obtain information of real value from an experiment of this sort, not only

must it be preceded by a thorough and scientific investigation of the conditions, both in respect of fly and of the trypanosomiasis of man, stock and game, but it must be followed by an equally careful investigation extended over a sufficient length of time—probably running into a number of years—or no precise information regarding the results of game elimination can be expected. In due course Dr. Lloyd will, doubtless, go on leave and then, if we can be guided by what usually happens on such occasions, the work will either come to an end, or, which amounts to the same thing, someone who has other interests, or no interests at all, will take over.

In connection with this game exclusion experiment, you will perhaps pardon me if I refer to a passage in the extremely interesting Report of the East Africa Commission which I had the pleasure of reading a few weeks ago. The passage runs as follows :—

‘ The question of game destruction is a very thorny one and has aroused much feeling. In this connection the opinion of Mr. Walter, now Lord, Rothschild, is worth recording : “ To prove to the utilitarians the absolute uselessness of this proceeding, I should like to point out that the extermination of the game animals in any large area would be a task of several years’ duration and the following would take place. As, year by year, the large animals grew scarcer, the tsetse flies *Glossina palpalis* and *morsitans*, which are the means of spreading sleeping-sickness in man and nagana in animals, would be driven to bite monkeys, carnivora, rats, mice, and the numerous small animals of those regions ; these would be infected and the trypanosomes of the disease would gaily survive. This would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases.” ’

Now, sir, I must confess that personally I should have experienced some difficulty in finding anything less worth recording. In the first place, Lord Rothschild ventures to prejudge in the most categorical manner, and without the slightest evidence, what would happen as the result of an experiment—to my mind a most dangerous and unwarrantable procedure—and in the second place, even assuming his premise, for which of course we have similarly no support, namely, that tsetse flies in the absence of large animals would be forced to feed on monkeys, carnivora, rodents and mice, the inference that ‘ these would be infected and the trypanosomes of the disease would gaily survive, and that this would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases,’ indicates complete

ignorance of what is known regarding the effect of the pathogenic trypanosomes of man and stock on these small animals.

I mention this because it illustrates in such an admirable manner my point that the Trypanosomiasis problem is not one which can be fully investigated by entomologists alone or, for that matter, by any other class of worker.

It is not my intention to refer in detail to the most valuable work which Dr. Duke is carrying out in Uganda on the protozoology and epidemiology of the disease. All who are familiar with his work realize its great value, but here again I feel that the work is suffering because Dr. Duke's other duties make great demands upon his time and because he lacks sufficient expert assistance and adequate financial resources to put to the crucial test the theories which he has built up at the cost of years of patient research. I am glad to learn that one of the recommendations of the International Conference on Sleeping Sickness which met in London last month under the auspices of the 'League of Nations' is that the International Commission, which it is suggested to form, should be placed under the presidency and control of Dr. Duke, and I am especially glad to see that they have coupled the recommendation with the suggestion that Dr. Duke's staff should be increased by the services of a bio-chemist and entomologist. Apart from this, I do not hope for much from the labour of the International Conference. Such a Conference seems to me to be premature. I cannot believe that its efforts are likely to advance knowledge and we hardly know enough at the present time to formulate regulations governing the International Frontiers in Tropical Africa. In my judgment, much more is to be hoped from an inter-Colonial Conference and from the co-ordinated and sustained effort which it would be in the power of such a Conference to ensure.

The case for co-ordination appears to me to be overwhelmingly strong, for the following reasons :—

Many investigators are at present working in more or less isolation at the different aspects—entomological, epidemiological, pathological, and therapeutic—of trypanosomiasis. The cost of this work is divided amongst many Colonies and therefore probably does not fall unduly heavily upon any one Colony, but in the aggregate the total annual expenditure must be very large. Unfortunately, as the

individual workers are isolated, insufficiently supplied with funds and assistance, and compelled to leave their work at more or less stated intervals, and sometimes at periods which are not stated, it is not surprising that much that is done is unsatisfactory and incomplete owing to want of organisation to ensure continuity, and consequently much time and money is wasted. Such a process has continued long enough; it is uneconomical and although the expense to each Colony may be relatively slight, in the aggregate it is large, and knowledge, if it advances at all, does so slowly and uncertainly.

Many of the problems which demand solution are very large, as, for example, the relationship of game to trypanosomiasis and the tsetse or the various problems which, five years ago, the Glossina Sub-Committee proposed should be investigated. Such problems cannot, with any hope of success, be investigated by isolated workers, whether entomologists or pathologists, but only by large and well-equipped Commissions having at command large funds.

I would therefore urge, as I did five years ago, (1) that in future, effort should be concentrated instead of dissipated; (2) that the work of the entomological and medical and veterinary research into the Trypanosomiasis problem be combined under one central organisation, and such organisation be supported by pooled contributions of all the African Colonies interested; (3) that the personnel of the investigating commission or commissions be large enough to ensure continuity of work in all directions, thus obviating interruptions due to such exigencies as illness or leave and preventing staleness and inertia, which is so likely to result from isolation; (4) that sufficient funds be placed at the disposal of the investigating commissions to allow of the employment of adequate native labour, so that experimental work can be undertaken on a sufficiently large scale, thus enabling the investigation of the relationship of fly and trypanosomiasis to game, and of the various problems enumerated in the Report of the Glossina Sub-Committee of the Imperial Bureau of Entomology, to be carried out in a satisfactory manner and with some reasonable prospect of success.

Whether such a scheme would cost more than is at present being spent individually by the different Colonies, I do not know. Certainly a large sum would be needed, and if this were not forthcoming the whole plan would collapse. Whether the problem is sufficiently

grave to warrant a large expenditure in a serious endeavour to find a solution, I will not attempt to discuss, as I am neither a politician nor a student of political economy, but judging from the valuable Report of the East Africa Commission to which I have already referred I gather that certain politicians and economists are abundantly satisfied of the gravity of the situation.

A NOTE ON MICROFILARIAE IN TANGANYIKA TERRITORY

BY

J. F. CORSON

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PREVIOUS OBSERVATIONS

Geographical distribution and incidence. Little appears to be known of endemic areas. Feldmann (1904), Marshall (1909) and Grothusen (1910) noted the prevalence of *Mf. perstans* in the district of Bukoba. It had been observed previously by Zupitsa, in 1897-98. Feldmann examined over 6,000 persons and found from 24 to 86 per cent. of the population, in various parts of the district, infected, the northern parts showing a greater incidence than the southern. In the south-east of the territory the district around Liwale appears also to be an endemic area as *Mf. perstans* was found by Mr. Irvine, sub-assistant surgeon, in 1924, in 31.5 per cent. of 402 adults examined. In regard to *Filaria bancrofti* even less is recorded. Elephantiasis was stated by Grothusen (1909), and previously by others, to be endemic in the Ukena-Ebene area, to the north-west of Lake Nyassa. Recent reports point to the prevalence of this disease in districts near the great lakes, as Mwanza and Bukoba in the north, and near Tukuyu (Neu Langenburg) in the south. In the region of the sea coast, there appear to be many cases of elephantiasis in Mafia Island, though no figures are yet available, while a considerable number are operated upon in the larger towns. Engeland (1920) found 32.32 per cent. of 297 native soldiers at Dar-es-Salaam infected with *Mf. bancrofti*. They, however, like the population of the coast towns, include people from many different parts.

Incidence in Dar-es-Salaam. In the years 1908-09 and 1909-10, microfilariae were found in slightly over 2 per cent. of nearly 40,000 thick blood films, examined chiefly for malaria and taken, no doubt, in the day-time. During the years 1922, 1923 and 1924, in the course of routine examination of a large number of thick blood films at the bacteriological laboratory at Dar-es-Salaam, microfilariae,

sheathed and unsheathed, grouped together, were found, as recorded in the annual reports, in 2.5 per cent., 2.2 per cent., and 3.6 per cent., respectively. The much greater incidence of *Mf. bancrofti* than of *Mf. perstans* in such examinations, was noted by Engeland and Manteufel (1911).

Species of microfilaria found. *Mf. bancrofti* and *Mf. perstans* only were found by Engeland and Manteufel. Fülleborn also (1908 and 1913a) found only these two species, with one possible exception, in blood slides received from Bukoba, Usumbura and Shirati, near the great lakes in the north, and from Dar-es-Salaam. The possible exception was a very small sheathed microfilaria, found by Manteufel (1911) in the blood of a soldier's boy in Dar-es-Salaam. The slide sent to Fülleborn, broken on the way, contained only about 10 microfilariae, stained with haemalum. Fülleborn admitted, with all reserve, that it might belong to a new species or be *Mf. powelli*, but that its possible identity with *Mf. bancrofti* could not be excluded. *Mf. loa* has not been found. Neave (1912) gives a list of flies found in German East Africa, which includes three species of Chrysops, viz., *C. bicolor* Cordier, *C. longicornis* Macq, and *C. magnifica* Austen. Limited reference only to subsequent literature has been available and this may not represent present knowledge. It is of interest that a considerable number of natives of West Africa were brought to this country during the late war.

Periodicity of Mf. bancrofti. That well-marked periodicity occurs in at least a great majority of cases was shown by Engeland. He found the microfilaria, as stated above, in 32.32 per cent. of soldiers, in the night blood, but in only 2.06 per cent. were they present also in the day time. Whether or not forms without periodicity also occur, as in the Southern Pacific and, perhaps, in West Africa, has not apparently been shown.

PRESENT OBSERVATIONS

From 300 to 400 thick blood films, chiefly of Africans, but including some Indians, taken at various unstated hours of the day, are examined monthly in ordinary routine work at the bacteriological laboratory at Dar-es-Salaam. In 768 such films, examined recently, microfilariae were found in 6.7 per cent. The latter figure refers to different individuals so the percentage given is a minimum.

Similar films were taken between 10 and 11 a.m., from 140 school pupils and from 140 prisoners. In the former, *Mf. bancrofti* was found in eight cases (5.7 per cent.) and *Mf. perstans* in one. In the latter *Mf. bancrofti* was present in seven cases (5 per cent.) and *Mf. perstans* in five. Examination in detail, including measurements with a camera lucida, was made in 30 cases, and observations as to periodicity in 21 cases. In a few cases only were living specimens examined and 'vital' staining with neutral red and azur II used. Staining with weak methylene blue, as described by Foley (1913) and by Sharp (1923) was not employed. Giemsa's stain and haemalum were chiefly used. The results confirm those of German observers. Microfilariae indistinguishable from *Mf. bancrofti* and *Mf. perstans* were the only kinds observed. Brief mention of a few details only is therefore made.

Mf. bancrofti. *Morphology*. The *sheath*, in nearly all the specimens, appeared to be unstriated. In one specimen the free anterior part of the sheath, in length about equal to one-fourth that of the worm, showed very clear, regular cross striation. If, as has been suggested by Fülleborn (1913b), it may be simply an impression of the striation of the body of the worm, in this case it was remarkably well-defined. Striation of the sheath has been observed by Brumpt (1922), who suggested that it might indicate a larval skin, and by Foley. It is referred to in a review of a paper by Biglieri (1923). The *anterior end* of the worm, in specimens stained with Giemsa's solution, showed various appearances, corresponding more or less with the descriptions of various authors. An interpretation of them could not confidently be made. Neither in living nor in stained specimens could a 'fang' or 'prepuce' be recognised. *Striation of the body*, in deeply-stained specimens, was observed to extend throughout the whole length of the worm, from the extremity of the anterior end to the tip of the tail. With ordinary staining it was not seen in front of the first nuclei. The *nuclei* were counted in a few specimens. The results did not agree with the figures given by Sharp. The number in front of the 'nerve ring,' for example, was from 60 to 70 or more. The appearances of the '*excretory cell*,' the '*central viscus*' or '*Innenkörper*' and the '*G¹ cell*' of Rodenwaldt agreed with Fülleborn's description and illustrations of *Mf. bancrofti*. The *tail* was not infrequently folded upon itself.

Measurements. 114 specimens from 26 cases. As the tip of the tail could not be seen clearly in many specimens, the *last tail nucleus* is assumed to be at 95 per cent. of the total length. The average position in 17 specimens measured was at 94.6 per cent.

The terms denoting the 'fixed points' of Fülleborn are used.

| N. | Ex-P. | Ex-C. | G ¹ -C. | L. Tail-C. | Total length. |
|----|-------|-------|--------------------|---------------|------------------|
| 19 | 29 | 29.5 | 69.7 | 272.8 | (287.1) |

In over 83 per cent. of 114 specimens the '*nerve ring*' was situated at a point between 18 per cent. and 20 per cent. of the length. The following are the figures of Fülleborn's measurements of 28 examples.

Microfilariae from German East Africa; ordinary thick dry preparations from 5 different slides; haemalum staining.

| | N. | Ex-P. | G ¹ -C. | A-P. | L. Tail-C. | Total length |
|-------------|------|-------|--------------------|-------|---------------|-----------------|
| Average ... | 19.9 | 30.1 | 70.3 | 82.6 | 95.3 | 263.7 |
| Minimum ... | 18.3 | 27 | 67 | 79 ? | 94.4 | 245.5 |
| Maximum ... | 21.1 | 33 | 73.3 | 88.4? | 96 | 291 |

Periodicity. Only persons in whose blood microfilariae were found in the daytime were examined. Measured quantities, viz., 20 c.mm. of blood were taken in 13 cases. In eight other cases, by the kindness of the staff of the native hospital, two thick films at midnight and two by day were taken on two or three successive days. The day time was usually 9 a.m. In no case was the number of microfilariae in the day blood equal to that in the night and in nearly all cases the disproportion was greater than could be accounted for by ordinary fluctuations, at the same hour on successive occasions, of, say, 300 per cent. Fluctuations are known to be considerable. In one case, for example, the number in 20 c.mm. of blood taken at the same hour of the night on several occasions, varied between 235 and 604. This case showed 17 at 5 p.m. and one only at noon, on two occasions. The greatest number found in daytime blood was 62, in 20 c.mm. of blood at 9 a.m. In this case, at 11 p.m., the number was 211. One case showed seven at noon and 13 at 11 p.m. So far as any conclusion can be drawn from these few observations, it would appear that a form of *Mf. bancrofti* without periodicity, if existing at all in this country, must be rare.

Mf. perstans. Citrated blood was centrifuged and dehaemoglobinised, for examination, especially of the anterior end.

In living specimens a small spot, usually terminal, occasionally apparently lateral, was seen. 'Vital staining' with neutral red showed a small conical structure, base to the front, just behind the outline of the anterior end. In some specimens the anterior margin appeared to show minute papillae. No constant feature, however, could be seen in the examination of about 20 specimens. No 'fang' was seen.

Stained specimens. These showed the morphological characters of *Mf. perstans*. The nuclear 'break' at 84 per cent. was a fairly constant character. Deep staining failed to show more than very slightly-marked cross striation.

Measurements. 47 specimens were measured. There is some lack of uniformity in measurements given by different authors even in the position of such a constant and sharply-defined feature as the 'nerve ring.' The following examples are given for comparison.

Brumpt (1922). Fülleborn's terms are used.

| N. | | Ex-P. | | A-P. |
|------|-----|-------|-----|------|
| 26.4 | ... | 36 | ... | 83 |

These figures are quoted by Stephens and Yorke (1921).

Rousseau (1919).

| N. | | Ex-P. | | G ¹ -C. | | A-P. |
|----|-----|-------|-----|--------------------|-----|------|
| 25 | ... | 32 | ... | 62.6 | ... | 83.5 |

Macfie and Corson (1922).

| N. | | Ex-P. | | G ¹ -C. | | A-P. |
|------|-----|-------|-----|--------------------|-----|------|
| 22.5 | ... | 32.7 | ... | 62.3 | ... | 81.1 |

In the present cases the average figures were as follows :—

| N. | | Ex-P. | | A-P. | | Total length. |
|------|-----|-------|-----|------|-----|---------------|
| 22.8 | ... | 31.9 | ... | 84.2 | ... | 189.2 |

In over 90 per cent. of the specimens the position of the 'nerve ring' was between 20 per cent. and 24 per cent. of the length.

The small form of *Mf. perstans* was not observed.

SUMMARY

1. Little is known of endemic areas of filarial infection in Tanganyika Territory. In addition to districts near the great lakes in the north-west, *A. perstans* is probably endemic in the south-east, around Liwale.

2. *Mf. bancrofti* and *Mf. perstans* are the only forms known to occur.

3. *Mf. bancrofti* was found to have a well-marked periodicity in all cases in which sufficient numbers were present, though occurring, in a small proportion of cases, in the blood in the daytime.

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SPRING RELAPSES IN BENIGN TERTIAN MALARIA

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While engaged, between 1916 and 1918, in the treatment of soldiers who suffered from malaria and who had been invalided from the Salonika army to the hospital base at Malta, I was struck by two clinical facts. The first was that patients who had been transferred to convalescent camp after recovery from attacks of benign tertian malaria, showed an extraordinary tendency to have relapses in the early spring, although the weather was genial; the second, that several patients whom I had treated (1919) in the late autumn, for severe subtertian malaria, remained well for two months or longer, and then relapsed about February, but this time with a benign tertian infection. It is possible that the men referred to in the latter case had previously experienced attacks of this form of malaria, although, in some instances at least, there was no history of it. It seemed possible that the tertian infection remained latent for some months after inoculation of the subject, and showed itself for the first time some months later. Indigenous malaria is extremely rare in Malta, and one could be all but certain that the tertian infection had not been acquired on the island, but there was no certainty that men coming from such a highly malarious country as Macedonia had not previously suffered from a benign tertian attack.

Acton, Curjel and Dewey (1921) record a similar experience. Of a series of 102 malignant tertian infections received for treatment at Dagshai, in India, in 1918, only seven were diagnosed as mixed parasitic infections due to the benign and malignant tertian parasites; yet in this series there were 64 benign tertian relapses, indicating that the majority of these mixed parasitic infections had been overlooked.

Some time ago a patient of my own developed benign tertian malaria in a manner which leaves little doubt that the first attack of the disease occurred after an incubation period of several months.

W.A., aged 37, a ship's officer, consulted me on 2nd December, 1921, about an illness which had been diagnosed as malaria. His ship had been in Bombay for a fortnight, from 1st October, 1921, and just before she left port he fell ill with malaise and headache, but without rigor or vomiting. This illness ceased in three or four days. A fortnight later, on the voyage to Europe, he had a second attack, of four days' duration, with similar symptoms. A third attack occurred about 7th November, while his ship was lying at Antwerp, and then for the first time he had shivering and some vomiting. When I saw him four weeks later, he felt out of sorts, but had 'on days and off days.' The spleen was considerably enlarged, and subtertian parasites, both rings and crescents, were present in the blood.

He was given a thorough course of quinine, beginning with a daily intravenous injection of bihydrochloride for four days, the first of 10 grains, and the three others of 15 grains each. From 7th till 26th December he took, by the mouth, 30 grains of quinine sulphate daily in solution; thereafter, for a month, 20 grains a day; and then 12 grains daily. A few crescents were present in the blood on 9th December. On seven subsequent examinations, up to 10th February, no parasites were found. By the middle of January the patient felt very well, and remained well, still taking 12 grains of quinine sulphate daily, until 8th March, when he vomited in the evening, complained of headache, and shivered a little. Two days later he had a more severe attack, and a two-day periodicity was established when I was called by his doctor to see him on 15th March. On that date the spleen was two inches below the ribs, and parasites, now benign tertian, were numerous in the blood. Subtertian forms were not observed, and the patient said that the symptoms were quite different from those which he had previously experienced, the shivering and headache being severe, and the attacks periodic. He quickly improved with a further course of treatment, but relapsed on 15th June, when benign tertian parasites were again found in the blood. There was a further relapse at the end of September, after

which he remained well until he passed out of my observation in January, 1923.

In this case there was no history of previous benign tertian malaria, and as the period from December till March was spent in the west of Scotland, infection must have taken place at least five months before the first attack. His previous visit, before October, 1921, to a malarious country was in March of the same year, when his ship had called at Bombay. But his first malarial attack of any kind did not occur till October, 1921. The subtertian parasites were apparently killed off by the course of quinine, but the benign tertian resisted it and caused an attack, even although the patient was taking 12 grains of quinine sulphate a day. It is to be noted that this tertian attack came on in the spring, as in the cases I referred to in the opening paragraph.

Although no reference is made to this peculiarity of tertian malaria in most text-books, the point is not a new one, for Dr. J. G. Jack has drawn my attention to a passage in Shakespeare's *Henry V*, Part 1, Act 4, Scene 1, lines 111-112, where Hotspur says :

‘ No more, no more ; worse than the sun in March,
This praise doth nourish agues.’

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ANTI-RABIC PROCEDURE IN PALESTINE WITH SPECIAL REFERENCE TO DECEN- TRALIZATION OF TREATMENT

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I. INTRODUCTION

Rabies has been known to be endemic in Palestine for very many years. The country, in virtue of its geographical position, is one peculiarly liable to the disease in that it is bounded on three sides by countries in which rabies is prevalent while its general configuration favours the continued existence of the common 'carriers' of the disease—the jackal and the pariah dog.

In the decade immediately prior to 1923 in Palestine, the most convenient course open to persons bitten by animals suspected of rabies was to proceed to Jerusalem to attend for treatment at a private institute where Pasteur's original dried cord method was practised.

On the establishment of railway communications between Palestine and Egypt in 1918, however, the Army Authorities made the decision that all military personnel affected should be sent to the Anti-Rabic Institute in Cairo.

This somewhat unsatisfactory state of affairs continued until it became increasingly apparent that the necessity for the provision in Palestine itself of a mode of treatment, recognized as adequate both by Military and Civil Authorities alike, was absolute, if numbers of lives were not to be sacrificed every year. Such loss of life was ascribed to two causes: to lack of proper facilities for treatment in this country, and to ineffective treatment in Cairo consequent upon the lateness of arrival of the patients.

Early in 1923 the serious attention of the Department of Health was given to coping with the situation ; there was no doubt that persons in ever-increasing numbers were being bitten by rabid dogs and jackals all over Palestine ; the result was that an undertaking to supply an anti-rabic vaccine of undoubted reputation and of proved reliability,—one able to fulfil all army and civilian requirements—was given by the Laboratory Section of the Department.

Table I shows the number of untreated cases reported on as having died in hospitals during 1922 from Hydrophobia, and well illustrates the gravity of the situation at that time.

TABLE I.

| No. of cases | Sex | Age | WOUNDS [(all inflicted through naked skin and not cauterized)] | | | Biting animal | In days | |
|--------------|-----|-----|---|--------------------------|---------|---------------|-------------------------------------|----------------------|
| | | | No. | Location | Gravity | | Period of incubation of hydrophobia | Duration of symptoms |
| 1 | M | 13 | 1 | Outer surface lower leg | Slight | Dog | 38 | 4 |
| 2 | M | 13 | 1 | Finger | Slight | Dog | 22 | 2 |
| 3 | M | 78 | 3 | Dorsum of hand | Severe | Dog | 44 | 4 |
| 4 | M | 47 | 3 | Eyebrow, Nose, Upper Lip | Severe | Jackal | 16 | 3 |
| 5 | M | 22 | 2 | Hand | Slight | Dog | 30 | 3 |
| 6 | M | 12 | 2 | Ala and tip of nose | Medium | Dog | 17 | 1 |
| 7 | M | 8 | 1 | Tip of nose | Medium | Dog | 44 | 2 |
| 8 | M | 45 | 2 | Nose | Medium | Jackal | 32 | 4 |

Additional considerations, however, emphasised the necessity for the undertaking, not the least amongst which was the fact that all expenses in connection with indigent patients presenting

themselves for treatment at the private institute in Jerusalem had to be defrayed by the Government at very considerable cost, while again, the Military Authorities were involved in much inconvenience and expenditure by having to send all bitten soldiers to Egypt.

It will be appreciated, therefore, that the Department's decision was based on strictly utilitarian and economic grounds.

II. SELECTION OF VACCINE

As the result of a lengthy and comprehensive survey of the various recognized modes of anti-rabic treatment, we finally resolved that the vaccine most suitable for Palestine was undoubtedly that originally introduced by Fermi of Sassari, modified by Semple, and elaborated by Harvey and McKendrick at the Central Institute, Kasauli.

The valuable reports issued by the Kasauli Institute, along with the published memoirs of Semple, on the one hand, and the close study, on the other, of the economic conditions of Palestine determined our selection.

The relative values of the three chief methods of treatment—all of which had received fair and full trial at Kasauli—are clearly enunciated by Harvey and Acton in their critical 'Examination into the degree of efficacy of anti-rabic treatment,' while the reasons which led up to the final adoption by that Institute of a carbolized vaccine in preference to the living viruses of Pasteur and Hoegyes are stated in convincing fashion.

After the inception of the Institute in 1900 the dried cord method of Pasteur was employed for seven years. While, admittedly, this treatment was highly successful, yet the occurrence, though rare, of 'accidents paralytiques' both during and after treatment could not be regarded otherwise than with disfavour. The possibility, however remote, of such accidents—believed by Harvey and McKendrick to be probably of an anaphylactic nature and due to the unavoidable introduction by this method of an excess of nerve protein during the course of injections—was responsible for a change being made to the dilution method of Hoegyes in 1907. This alteration in policy was in every way justified and the results of its adoption afforded striking confirmation of the theory advanced

by Harvey and McKendrick, for with the use of a comparatively small amount of foreign nerve protein coincided the complete cessation of cases of paralysis.

A further change of policy, however, took place in 1912 when, as a result of brilliant researches by Fermi, Semple and others, it was conclusively proved that the employment of a dead vaccine afforded a protection at least equal to that given by the living viruses of the previous methods.

Since that time the accumulated evidence of twelve years' experience has upheld beyond all question the opinion first put forward by Fermi as to the value of treatment by a carbolized vaccine.

Again, the stimulating example set by Col. Hamerton who, faced with an exceedingly difficult problem in Irak, most successfully coped with the situation there, proved a great encouragement to us, and so it was with every hope of similar success in Palestine that we established in Jerusalem, on May 1st, 1923, the Central Anti-Rabic Institute. In order, however, that the 'greatest good of the greatest number' of inhabitants likely to be at risk might be served, ten subsidiary treatment centres in the districts, supplied with the vaccine prepared at the Central Institute, were opened on the same day.

III. PROCEDURE

It will be immediately obvious that if decentralization is to be successful, medical officers in charge of subsidiary centres must be completely conversant with all matters relating to anti-rabic procedure.

Each officer appointed to carry out such work here reports first to the Central Institute where he undergoes a full course of instruction.

During this course he has to give evidence that he has familiarized himself with the various considerations governing the policy adopted, with the whole subject of rabies and its prevention, and with every detail of procedure touching on the preparation, distribution, storage, and administration of the vaccine.

To ensure that each centre is conducted in strict accordance with instructions laid down, one or other of us carries out routine inspections.

A. GENERAL CONSIDERATIONS.

(1) *Was the biting animal rabid?*

An animal should be considered rabid

- | | |
|--|--|
| (a) if it dies from an undiagnosed disease | } within ten days of its biting the patient. |
| (b) if it has been killed. | |
| (c) if it has disappeared | |
- (d) if it shows marked alteration in behaviour; if, for example, it makes unprovoked assault on human beings or other animals, especially if such results in many persons or animals being bitten.
- (e) if a jackal makes an unprovoked attack on a human being.

(2) *Has the patient been exposed to the risk of infection?*

(a) A person cannot be infected by (the saliva of) an animal except when that animal is actually suffering from rabies and during the ten days immediately prior to that animal's developing symptoms (two to five days before the appearance of symptoms, Roux and Nocard, Nicholas).

(b) The contagion, no matter what its virulence or concentration, cannot penetrate uninjured skin or mucous membrane.

In this connection, wounds which have been granulating twenty-four hours or more are considered impervious to the virus.

A very definite risk of infection is run when blood or serum oozes from cut or abrasion.

B. PROCEDURE TO BE FOLLOWED.

(1) *In respect of the biting animal.*

(a) On no account should the animal be destroyed (there are, of course, necessary exceptions to this dictum). It should be kept under observation in close confinement, when it will, if infected and infective, have developed symptoms within ten days.

If, on the other hand, the animal remains healthy at the end of ten days, it can be regarded as having been free from the possibility of causing infection at the time of biting.

(b) If the animal dies during the period of observation (ten days) without showing the classical signs of rabies, it should, nevertheless, be considered as having been rabid in so far as the treatment of bitten persons is concerned.

(2) *In respect of the bitten person.*

(a) After giving suitable local treatment to the wound (*vide* below), one must pronounce upon the biting animal. If the animal appears to be normal it should ordinarily be kept under observation for ten days, at the end of which period, if it still shows no signs of disease, it can be considered to have been non-infective and the bitten person therefore free from risk. But treatment should be begun immediately (and to this there must be no exception made), in the following circumstances :—

(a) When the animal develops symptoms of ill-health, dies, is killed, or escapes, during its ten days' observation period.

(b) When the patient has been bitten on the face, on the hand, or very severely (even through clothing) in other parts of the body.

(c) If the patient has been bitten by an unknown animal in the dark or while asleep in the fields (common incident enough during the summer among the *Pellaheen*).

It is, however, of first importance to note that when an animal dies during its observation period, vaccine treatment must be begun at once, and on no account whatsoever should the result of the laboratory examination of the dead animal's brain be awaited. Apart from the loss of valuable time incurred by such a wait, it must be remembered that failure to find Negri-bodies by the microscopist does not always signify that the animal was free from rabies.

(3) *In respect of the bitten animal.*

See Appendix II, paragraphs 5 and 6.

C. DIAGNOSIS OF CANINE RABIES.

(1) *Clinical.*

There exists at present no better description than that given by Mohler and Eichorn in their translation of Hutyra and Marek's textbook on '*Spezielle Pathologie und Therapie der Haustiere*,' published by Ballière, Tindall and Cox, and to this the reader is referred.

(2) *Laboratory.*

The brain of a dog or other animal which has died of suspected rabies or which has been shot or otherwise killed as a result of its

having bitten persons or other animals should be extracted and forwarded to the Central Institute (here part of the Central Laboratories). A simple method of extraction is to saw the head sagittally and remove the two halves of the brain intact.

When the brain has to be sent from a distance for laboratory examination, the two halves, so extracted, are placed in a wide-mouthed can and packed about with well-powdered salt. The salt must completely fill the receptacle. The lid is now to be replaced and soldered by a tinsmith prior to despatch.

We advocate this procedure of forwarding in salt in preference to that usually recommended, viz.: in separate tins containing glycerine and alcohol respectively, because the brain thus arriving in a fresh state allows of full examination.

The brain is now freed from salt by washing in sterile normal saline solution.

Laboratory investigation consists of the search for Negri-bodies and of the performance of the biological test by rabbit inoculation.

For the detection of Negri-bodies, preliminary smears from the hippocampus major or cerebellum are made and coloured with Giemsa's stain. The results are controlled by examination of sections of these parts of the brain fixed in Zenker's fluid and coloured with haematoxylin-fuchsin or Mann's stain. With the latter we have found difficulty in obtaining uniformity of results, but with haematoxylin-fuchsin we have been very successful in accordance with the following procedure :—

- (a) Stain with haematoxylin for seven to ten minutes.
- (b) Blue well in tap water.
- (c) Counterstain with acid fuchsin for three minutes.
- (d) Differentiate in tap water.
- (e) Pass rapidly through alcohol, clarify with oil of cloves, and mount in balsam.

In regard to the Biological Test: it is performed by injecting an anaesthetized rabbit sub-durally with 0.2 c.c. of a 1 per cent. emulsion previously prepared from the medulla of a suspected animal.

The emulsion is made by rubbing-up in sterile normal saline solution a small piece of the medulla and thereafter filtering the suspension through gauze. It is then introduced sub-durally by

means of a hypodermic syringe (the needle of which is bent at a right-angle) into an opening made by a small (Eyre's intracranial) trephine in one of the parietal bones of the rabbit. It need hardly be pointed out that the strictest aseptic precautions must be observed throughout the operation. After a variable length of time in positive tests symptoms of paresis occur, and death takes place usually in from fifteen to twenty-five days.

D. TREATMENT.

When it has been decided that a patient requires treatment certain curative methods are employed.

(1) *Local treatment.*

Every endeavour must be made to get rid of as much of the virus deposited in the wound as possible and that without delay. Such common expedients as ligaturing, when possible, the affected part, encouraging bleeding, and freely opening up and thoroughly cleansing the wound are to be resorted to.

Cauterization of every part of the wound must now be performed with such caustics as pure carbolic or fuming nitric acid. Here we employ fuming nitric acid and consider it likely to be effective only if applied within half-an-hour of the time of biting. (Adherence to this time limit enables us to state that in our series of cases, of 1,920 persons treated, only 40 were efficiently cauterized.)

(2) *General treatment.*

The patient must, during treatment and for ten days thereafter, follow a quiet well-ordered existence. Chill, fatigue, and excitement must be avoided, while alcohol should be especially restricted.

(3) *Specific treatment* (vaccine treatment).

E. THE VACCINE.

(1) *Nature and Preparation.*

The vaccine prepared by the Central Institute consists of a 2 per cent. suspension of the brain of a rabbit killed with fixed virus, in a solution of 1 per cent. phenol in distilled water.

Before being bottled and issued to the ten treatment centres, however, it is diluted with an equal quantity of normal saline

solution. Rabbits of about 1,400 grammes are inoculated sub-durally with fixed virus emulsion, and this operation is performed only on such numbers of rabbits as will presumably supply all demands for vaccine and will keep the 'strain' going.

Attention to the technique of the trephining and inoculating operations results in a minimal number of rabbits being required, and the practically 100 per cent. success obtained here is due to the operators' observing various essentials :

- (a) Absolute asepsis throughout.
- (b) Rapidity in carrying out the trephine portion of the operation.
- (c) Slowness in injecting the emulsion of fixed virus along the needle, held parallel to the dura.

(d) Covering the trephine opening with a pad of sterile cotton wool and maintaining slight pressure during the process of inoculation and during the slow drawing-out of the needle on completion of the operation. This procedure precludes regurgitation of the virus emulsion.

- (e) Care in the choice of the fixed virus used for passage.

The virus in use here was originally obtained from Cairo and produces symptoms usually on the fifth, though occasionally on the sixth day after sub-dural inoculation. Whereas, for the production of vaccine, the brain of any animal showing symptoms on the fifth or sixth day is used, here 'passage virus' is invariably selected from the brains of those rabbits that have developed symptoms on the fifth day.

This we find most important in keeping up the fixity of the virus and in obtaining uniform results of virulence.

On the eighth or ninth day, then, after inoculation, the rabbits, now moribund, are killed by chloroform, dipped in a weak solution of cresol, and dissected.

The brain, after naked-eye inspection and after cultures have been taken from it to ensure sterility, is 'extracted' and weighed in an accurate balance. Brains showing excessive haemorrhage or other abnormality are discarded. The brain is now pounded in a sterile mortar and during this process the carbolic solution is added little by little. On an average this operation should take from fifteen to twenty minutes to complete, and when the brain has been well-emulsified into a thick sticky paste, the remainder of the

carbolic solution is slowly mixed in, until a suspension of 1 in 50 of brain substance has been obtained.

We now have a 2 per cent. brain emulsion in a 1 per cent. carbolic solution and this is filtered through two layers of gauze to get rid of the connective and vascular tissues, and then placed in an incubator at 37° C. for twenty-four hours.

Although all workers whose methods we have studied use a carbolic solution made up with normal saline, we, after repeated observation, have preferred to employ distilled water, as this we find gives a better suspension, while precipitation of the brain matter does not occur so readily as with saline.

After the emulsion has been in the incubator for twenty-four hours, it is taken out and mixed with an equal volume of normal saline so that the vaccine now consists of a 1 per cent. brain emulsion in 0.5 per cent. carbolic solution. After passing aerobic and anaerobic cultivation tests, the vaccine is run into sterile bottles of 30 c.c. capacity in which it is distributed. Of this vaccine each person, irrespective of age, sex, or severity of bite, receives intracutaneously 5 c.c. daily, 2.5 c.c. on each side of the abdomen.

In the first year of work here, we diluted the above suspension further with an equal quantity of normal saline just before its administration, thus following the exact procedure (apart from the substitution of distilled water) described by Harvey and McKendrick and Col. Hamerton, and giving a 0.5 per cent. suspension of brain substance in 0.25 per cent. carbolic solution. In our second year, for considerations elsewhere discussed, it was decided to inject directly, without previous dilution, a 1 per cent. emulsion—and this has been in practice here since May, 1924.

(2) *Distribution, and instructions for use.*

Before despatch from the Central Institute the vaccine bottles are properly capped, paraffined, and labelled. On each label is given the following information: nature of the contents of the bottle; the dosage; the mode of administration; and directions relating to storage. In addition to this, by means of a rubber stamp are affixed further particulars:—viz., the serial number of the vaccine, the date of manufacture, and the date on which the vaccine bottle—irrespective as to whether its contents have been used or

not—is to be returned as 'out of date' to the Central Institute. This date of return is invariably three months after the date of manufacture shown on the label of each bottle.

The medical officers in charge of the various treatment centres make known their requirements fortnightly, and these indents, when met, maintain the stock held by each centre at a constant level.

The vaccine is administered in accordance with the printed instructions wrapped round each bottle :—

(a) Sterilize 5 c.c. Record syringe.

(b) Shake the bottle well.

(c) Withdraw 5 c.c. of vaccine by pushing the needle of the syringe through the rubber cap (previously sterilized by dipping in alcohol).

(d) Inject 2.5 c.c. of the vaccine on one side of the abdomen and 2.5 c.c. on the other, by inserting the point of the needle at an acute angle between the superficial and deep layers of the skin (intracutaneously).

(e) The complete course of treatment consists of fourteen such injections of 5 c.c. on successive days.

(f) The same dose is given to children as to adults, and bites of all severities are treated alike.

F. ADVANTAGES OF ADOPTING THIS METHOD OF TREATMENT.

(1) The employment of this carbolyzed anti-rabic vaccine precludes the possibility of its producing *per se* the disease its object is to prevent.

Cases recorded in literature show that the injection of living or merely somewhat attenuated virus is not entirely free from danger. Babes in this connection states: 'It is very probable that fixed virus in certain cases can produce hydrophobia after subcutaneous injections. The employment of this virus, therefore, must be prohibited in the treatment of human subjects unless the organism has been first prepared with a virus sufficiently attenuated.'

Surprising, indeed, is the number of persons requiring assurance that treatment itself involves no risk, and here one is frequently asked whether, should events prove that treatment was unnecessary, any harm can possibly ensue from the administration of vaccine.

With the use of carbolized vaccine, we are fortunately able to reassure patients completely in these respects.

(2) Its use is not followed by any harmful effects.

Occasionally local hyper-reaction occurs, due to individual idiosyncrasy. Here there has been no sign of abscess formation although over 60,000 intracutaneous inoculations have been made. Further, the vaccine contains the smallest amount of nervous tissue commensurate with efficient treatment, and thereby are avoided the so-called 'post-treatment paralyses' which occasionally follow certain other methods of treatment.

(3) *Accuracy of dosage.* That uniformly successful results have been established can be claimed for no method unless dosage of vaccine can be accurately determined.

In regard to cord methods two factors must be taken into account, which militate against an accurate estimation of the number of immunizing units injected into a patient.

(a) Cords differ much in size, varying largely in proportion to the size of rabbit employed. A very appreciable difference must exist between the numbers of immunizing units contained in 1 c.c. of thick and in 1 c.c. of thin cord, respectively.

(b) Where the dried cord method is used, it will be obvious that a ten days' dried cord, from a large rabbit, will contain, normally, more living virus than a ten days' cord from a small one because desiccation proceeds more rapidly in the latter. Such difficulties do not obtain with carbolized vaccine. If care be taken to pound the brain, during manufacture, for a fixed period, say twenty minutes, and to filter the resultant suspension through gauze of uniform thickness, emulsions of unvarying strength are produced.

(4) Its dosage over fourteen consecutive days (the complete period of treatment) remains constant for all bitten persons, irrespective of age, sex, severity of bite, location and multiplicity of wounding, interposition of clothing, and different conditions requiring consideration when other methods of treatment are employed.

The reason for the application of a universal dosage lies in the fact that each case for which treatment is prescribed is regarded as being sufficiently serious to warrant the full and intensive dosage given by this method.

(5) *Economy and rapidity of production.* The use of carbolized vaccine permits of great economy both in respect of animals and of time. From a rabbit of average weight (1,400 grammes) can be produced vaccine sufficient for twelve complete courses of treatment. Further, one does not require to inoculate rabbits daily, but only as occasion demands and just often enough to maintain the 'strain.'

The actual material cost of treatment of nearly 2,000 bitten persons, here, during the past two years, has been little more than the purchase price of 150 rabbits. The time taken to produce the vaccine is short and we have clearly shown that any well-equipped laboratory can, in addition to its routine work, undertake the successful manufacture of the vaccine without additional expert staff.

An illustration of the rapidity of production is afforded by a recent occurrence when, on a farm over 100 miles from the Central Institute, 75 valuable animals were bitten by a rabid dog.

The issue of a quantity of vaccine (5,250 c.c.) sufficient for the treatment of these 75 animals was made without delay, and within nine days our reserve stock was at its normal level.

(6) Carbolized vaccine retains its maximal potency and powers of immunization for a period of at least three months if preserved under requisite conditions—away from light and in an ice-box.

It is, consequently, suitable for use in small countries where rabies is present, but too rare to justify the expenditure involved in the creation of Anti-Rabic Institutes. Such countries can purchase, from time to time, quantities of vaccine sufficient for estimated maximal requirements and their stock can be renewed quarterly at small expense. Transjordan—a country adjoining and obtaining its vaccine supplies from Palestine—affords an excellent example.

On the other hand, when living virus is employed for treatment, vaccine cannot be sent to a distance without a diminution of efficacy and without risk of its becoming infected. It is for this reason that, in our belief, carbolized vaccine is the only one of practical value in the prophylactic and curative treatment of animals.

(7) The vaccine is manufactured in a Central Institute and can be issued therefrom to any number of treatment centres where its administration to bitten persons forms part of the routine duties of the Government medical officers there. The advantages to the bitten persons of this are as follows :—

(a) Treatment can be begun without loss of time—an incalculable advantage in a condition where the importance of the time factor is paramount.

(b) The patient can be treated at or near his own home, and thereby is avoided the necessity for his undertaking long fatiguing journeys.

(c) The bitten person may move from town to town in accordance with the dictates of his business and be assured of an uninterrupted course of treatment.

In this connection, and to demonstrate the exceptional applicability of this form of treatment to unexpected circumstances, we would refer to an episode which occurred during 1923. In a military camp at Sarafand, two British and five Indian soldiers were severely bitten by a jackal. The jackal was shot, its brain extracted and forwarded to the Central Institute, where the finding of Negri-bodies and a positive biological test proved the animal to have been rabid at the time of biting. The bitten persons were treated at Ramleh Anti-Rabic Centre for seven consecutive days, at the end of which time the Indian regiment was ordered to proceed abroad. The Regimental Medical Officer was supplied with vaccine sufficient for seven further injections to each patient, and the remaining half of the course was administered on board ship. Reports forwarded later showed that treatment had been successful in each case.

Probably one of the most satisfactory results of our procedure, however, is that no case of hydrophobia has occurred during the past year for lack of facilities for treatment. In marked contrast to this state of affairs were the conditions (summarised in Table I) obtaining in 1922—in the year previous to the adoption of the present system of decentralisation of treatment.

(8) *Efficacy. A.—Theoretical considerations.* It has to be admitted, however, that the only factor determining ideal treatment is its 'Efficacy.'

If a treatment is to be universally successful it has to be able not only to prevent the onset of hydrophobia in the case of average severity, but also to ward off the disease in grave cases of short incubation periods.

The greatest advantage claimed for carbolized vaccine and its

period of administration is that it is an attempt to deal with such cases as may have short incubation periods. There can be no possibility of cure once the virus has reached the brain ; now, in a certain percentage of cases the virus does actually attach itself to the brain in or in about fifteen days ; it is obvious, therefore, that any treatment calculated to prevent the disease in these cases must aim not only at producing in the system a sufficiency of antibodies, but at producing them *within fifteen days*. Under such circumstances the time limit is of first importance, and the logic of 'intensifying' treatments of head and face bites by prolonging them beyond the minimal period sufficient to excite and promote antibody formation in as great an area as possible is by no means clear. Surely here the thing to do is to increase the dosage administered within the minimum period of time at one's disposal. The importance of realising this cannot be over-estimated, and a glance at this modified table of Bauer (II) will show short incubation periods to be by no means rare. Further, in Table I, are two such cases.

TABLE II (after Bauer).

| Number of days incubation | Percentage of total |
|---------------------------|---------------------|
| 1-19 | 8.24 |
| 20-39 | 28.34 |

A short intensive course of treatment might likewise prove of extreme value in those cases where bitten persons report at an institute late. Here again time is all-important and the maximum dosage bearable should be administered in the shortest possible period.

Moreover, the use of brain matter instead of cord doubtless contributes towards the efficacy of carbolyzed vaccine and its superiority over cord methods.

Brain matter is said by Nitsch to be ten times more virulent

than spinal cord. In using brain, therefore, we are giving a larger proportion of specific antibody-producing substance and a smaller one of the useless, probably harmful, nervous tissue than is practised in methods of cord immunization.

B.—*Practical results.* We shall first record the experimental and then the practical evidence on which our assertions as to the efficacy of this method are based.

(1) *Experimental.*

The subjoining Tables III and IV are self-explanatory.

TABLE III
Immunizing Experiments.

| No. of experiment | Animal | Duration of Treatment | Quantity of 1% carbolized vaccine injected subcutaneously | Test | Result |
|-------------------|--------|-----------------------|---|---|-----------------|
| 1 | Rabbit | 14 days | 28 c.c. | 0.2 c.c. of 1% fixed virus emulsion introduced subdurally 15 days after last injection. | Lived |
| 2 | Rabbit | 14 days | 28 c.c. | | Lived |
| 3 | Rabbit | 14 days | 28 c.c. | | Died in 15 days |
| 4 | Rabbit | 14 days | 28 c.c. | | Lived. |
| 5 | Rabbit | 14 days | 28 c.c. | | Died in 18 days |
| 6 | Rabbit | Not treated | None | | Died in 8 days |
| 7 | Rabbit | Not treated | None | | Died in 8 days |
| 8 | Rabbit | Not treated | None | | Died on 9th day |

The temporary nature of the immunity conferred is well illustrated in regard to rabbit No. 2. Without prior immunization this animal was inoculated with 0.2 c.c. of 1 per cent. emulsion of fixed virus, fourteen months later, and died in eight days.

To show the evidence of immunity in the serum of rabbits treated with 1 per cent. carbolized vaccine, we have, guided by examples from existing literature, performed the experiments recorded in the next table.

TABLE IV.

| Animal immunized | Vaccine used for immunizing | Time after completion of treatment when serum was tested | Proportion of serum and 1% fixed virus emulsion tested | Tests applied to mixture of serum and virus | Result |
|------------------|-----------------------------|--|--|---|--------------------|
| Rabbit | 1% carbolized anti-rabic | 15 days | Serum 1 c.c. + fixed virus 1 c.c. incubated 2 hours at 37° | Subdurally into rabbit | Remained well |
| Rabbit | do. | do. | do. | do. | do. |
| Rabbit | do. | do. | do. | do. | Died 12th day |
| Rabbit | do. | do. | Only fixed virus 1% emulsion | do. | Died after 8th day |
| Rabbit | do. | do. | do. | do. | do. |
| Rabbit | Not immunized | Directly | do. | do. | Died in 8 days |
| Rabbit | do. | do. | do. | do. | do. |

(2) *Deductions from treatment of bitten persons.*

In the various records given below it has been considered advisable to include in the first part of each table, mostly for purposes of interest and comparison, the total number of bitten persons attending the various anti-rabic treatment centres; the number whose treatment was considered to have been unnecessary as reckoned by the biting animals having remained alive and well after ten days' observation, and the number of cases where a regular and complete course of treatment was carried out. On the last number alone have the death and so-called 'failure' rates been estimated.

The only fair and accurate means, however, of arriving at the actual success or failure of any line of treatment is to base the final calculations only on the number of bitten persons treated who had been definitely or presumably exposed to risk of infection.

The second part of each table, therefore, consists of an enumeration of :

(1) Those persons definitely-at-risk as proved by :—

(a) Laboratory investigation.

(b) Veterinary officer's certificate obtained after observation of the biting animal.

(2) Those persons presumably-at-risk, i.e., who had been bitten by animals fulfilling the conditions laid down under A. General Considerations, paragraph 1, items (d) and (e).

(3) Death and 'failure' rates calculated on a total of (1) + (2).

Statistics from May 1, 1923, to April 30, 1925.

Part 1.

| | |
|--|-------|
| Number of persons treated at various Government anti-rabic treatment centres in Palestine | 1,920 |
| Number of treatments interrupted or unnecessary | 470 |
| Number of regular and complete treatments administered | 1,450 |
| Total Deaths | 12 |
| Death rate | 0.82% |
| Deaths occurring later than 15 days after completion of Treatment | 4 |
| 'Failure' rate | 0.27% |

Part 2.

| | |
|--|-------|
| Number of persons definitely-at-risk— | |
| (a) Proved by laboratory investigation | 270 |
| (b) Proved by veterinary officers' certificates | 123 |
| Number of persons presumably-at-risk | 420 |
| Death rate | 1.47% |
| 'Failure' rate | 0.49% |

The above statistics, however, represent the results obtained from the combination of two definite work periods differentiated by alteration in daily dosage; thus, whereas in the period 1923-24, treatment consisted of the daily administration of 2 c.c. carbolyzed vaccine over fourteen consecutive days, during 1924-25 a dosage of 5 c.c. daily over the same number of days was substituted.

The results achieved during the two periods afford interesting comparison.

| | Period 1923-24 Dosage 2 c.c. of 1% emulsion | Period 1924-25 Dosage 5 c.c. of 1% emulsion |
|--|---|---|
| <i>Part 1.</i> | | |
| Number of persons treated | 886 | 1,034 |
| Unnecessary treatments | 138 | 332 |
| Regular and complete treatments | 748 | 702 |
| Total deaths | 9 | 3 |
| Death rate | 1.2% | 0.4% |
| 'Failures' | 4 | 0 |
| 'Failure' rate | 0.5% | 0 |

Part 2.

| | | |
|--|------|------|
| Number of persons definitely-at-risk— | | |
| (a) Proved by laboratory investigation | 180 | 80 |
| (b) Proved by veterinary officers' certificates | 48 | 75 |
| Number of persons presumably-at-risk | 213 | 207 |
| Death rate | 2% | 0.8% |
| 'Failure' rate | 0.9% | 0 |

It will be observed that 307 and 340 persons belonging respectively to the 1923-24 and 1924-25 periods are not included in Part II, although they have been bitten by animals fulfilling the conditions laid down in A. General Considerations, paragraph 1, items (a), (b) and (c)—conditions which demand the immediate treatment of the bitten persons from the point of view that the biting animals are to be considered rabid. Such number, if included, would obviously lessen both the death and 'failure' rate, thereby elevating the degree of efficacy of this form of treatment.

It is generally admitted that a certain percentage of cases cannot be benefited by any form of anti-rabic treatment. In such cases the incubation period is very short and the virus reaches the brain before a sufficient degree of immunity can be conferred. Excellent examples are afforded by numbers 2, 4, and 6, in Table I, and by the first line statistics of Table II.

On the other hand it must be admitted that when a patient dies of hydrophobia, say one, two, or three months after completing a full and regular course of treatment, this case cannot be included in the above category but must be considered as a failure of treatment in that either the effect of treatment was nil or that it sufficed merely to delay the arrival of the virus in the brain, thereby prolonging the incubation period of the disease, which latter alternative is exemplified in Table III, number 3 and 5, of our immunizing experiments.

Now in view of the results obtained in the 1923-24 period, with the administration of 2 c.c. daily for fourteen days, it may be a matter for surprise that we resolved to make an alteration in dosage to 5 c.c. daily over a similar period.

However, a study of the following four cases—our only failures of treatment as judged by the conventional standards, viz., when hydrophobia supervenes later than fifteen days after completion of a regular course of treatment—was mainly instrumental in bringing about our decision.

CASE I. Male, aged 10 years, severely bitten on forehead, hand and leg through naked skin by a jackal, reported one day after bite for treatment, the wound not having been cauterized before arrival. Treatment was regular over 14 days. Symptoms developed 59 days after the date of bite, and 44 days after completion of treatment.

CASE 2. Male, aged 35 years, severely bitten on face, eyelid, and thumb by jackal through naked skin, reported three days after bite, the wound not having been cauterized, and attended regularly for treatment. Symptoms appeared 81 days after the bite and 65 days after completion of treatment.

CASE 3. Male, aged 8 years, bitten slightly on upper extremity through naked skin by a dog, arrived two days after bite with wound not cauterized. Symptoms occurred 36 days after the bite and 21 days after completion of treatment.

CASE 4. Male, aged 30 years, bitten severely through clothing on lower extremity by a dog, arrived immediately after for treatment and had wound cauterized with fuming nitric acid. Treatment was administered regularly over 14 days. Hydrophobia supervened 39 days after the bite and 26 days after completion of treatment.

We felt convinced that these four cases died, not as the result of any special exaltation of a virus (*virus de rue renforcé*) or shortness of incubation period, but because the treatment administered had not been sufficiently intensive : with this conclusion Professor Fermi, of Sassari, to whom we submitted the facts, was in complete agreement. (It is, however, worthy of record that thirteen other persons, bitten by the animals inflicting bites on the four persons cited, received full and regular courses of vaccine also, and all thirteen are to-day alive and well, one-and-a-half years after completion of treatment.)

As a result an alteration in dosage was effected for all cases, on May 1st, 1924, after a month's preliminary trial had demonstrated beyond question the ability of children and adults alike to tolerate the daily increase from 2 to 5 c.c.

It was resolved by us to make an increase in daily dosage rather than in the period of vaccine administration, so that the method of treatment might be made as widely applicable as possible and especially with regard to such cases as might have short incubation periods.

Although we consider that results in human beings have justified the alteration, yet Table V will demonstrate our inability to adduce experimental proof of the value of increased dosage in laboratory animals.

Further, from this table it would appear that :—

(a) Better results in rabbits are obtained by the giving of small doses over a number of days (14) than of larger doses over a shorter period.

(b) It is impossible to reduce below a certain limit the time over which the total quantity of vaccine, ordinarily sufficient for complete immunization, can be usefully administered.

(c) As large a dose as 30 c.c. of a 0.5 per cent. emulsion in 0.25 per cent. carbolic may be given without the production of toxic symptoms.

TABLE V

| Experimental animal used | DOSE AND PERIOD FOR IMMUNIZATION | | | | | Whether or not animal survived treatment | SUBDURAL TEST 2 weeks after treatment tested with 0.2 c.c. of 1% emulsion of fixed virus |
|--------------------------|----------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--|---|
| | 2 c.c. daily for 14 days | 4 c.c. daily for 14 days | 6 c.c. daily for 10 days | 15 c.c. daily for 3 days | One injection of 30 c.c. | | |
| Rabbit | I | ... | ... | ... | ... | Survived | Survived |
| Rabbit | I | ... | ... | ... | ... | Survived | Died |
| Rabbit | I | ... | ... | ... | ... | Survived | Died |
| Rabbit | I | ... | ... | ... | ... | Survived | Survived |
| Rabbit | ... | I | ... | ... | ... | Survived | Survived |
| Rabbit | ... | I | ... | ... | ... | Survived | Died |
| Rabbit | ... | I | ... | ... | ... | Died | ... |
| Rabbit | ... | I | ... | ... | ... | Survived | Died |
| Rabbit | ... | ... | I | ... | ... | Survived | Survived |
| Rabbit | ... | ... | I | ... | ... | Survived | Died |
| Rabbit | ... | ... | I | ... | ... | Survived | Died |
| Rabbit | ... | ... | I | ... | ... | Survived | Died |
| Rabbit | ... | ... | I | ... | ... | Survived | Died |
| Rabbit | ... | ... | ... | I | ... | Survived | Died |
| Rabbit | ... | ... | ... | I | ... | Survived | Died |
| Rabbit | ... | ... | ... | I | ... | Survived | Died |
| Rabbit | ... | ... | ... | I | ... | Survived | Died |
| Rabbit | ... | ... | ... | ... | I | Survived | Died |
| Rabbit | ... | ... | ... | ... | I | Survived | Died |
| Rabbit | ... | ... | ... | ... | I | Died | ... |
| Rabbit | ... | ... | ... | ... | I | Survived | Died |

We would now draw attention to the fact that in the records of statistics published above have been included figures for total deaths and for the so-called failures of treatment as well as their respective percentages.

'Failure,' in accordance with convention, is applied only to

those cases which, in spite of treatment, develop hydrophobia later than fifteen days after a complete anti-rabic course has been administered. By the same convention are excluded from 'failures' all bitten persons dying during treatment or within fifteen days of its completion.

(Remlinger, e.g., has shown that during 1901-1908, in Constantinople, where the original Pasteur method is utilised, of persons dying despite treatment no fewer than 80 per cent. could be so excluded.)

Statistics based on this mode of computation will obviously vary with regard to :—

(a) Length of time taken to administer a course of treatment.

(b) Lateness of arrival of the bitten person for treatment.

The longer a person defers reporting for treatment, and the longer the period of treatment—the lower the 'failure rate' of an institute.

It is felt that such statistics tend unduly to emphasise the value of certain forms of anti-rabic treatment and especially of such as normally require a lengthy period for administration.

We realise that this method of recording deaths and failures would be ideal, if all institutes were to employ the same method whereby the same dosage of a similarly prepared vaccine was administered during the same period of time. As matters stand at present, however, when anti-rabic methods are many and diverse, it is our opinion that the only way to present statistics which can admit of real comparison is a simple enumeration of the deaths occurring at an institute and the percentage such bears to the total attending population proved or believed to be at-risk.

Deaths from hydrophobia should then be reported on in full, with especial reference to the duration of treatment, to the number of days intervening between completion of treatment and onset of symptoms, to the lateness of arrival for treatment, and to the irregularity of attendances during the course.

This information, in addition to that supplied in Appendix I, will generally enable an impartial pronouncement to be made upon such cases in their relation to treatment.

G.—Records. It was at once recognised that one of the criticisms most likely to be levelled against decentralisation of treatment would be the presumed unreliability of the records of available information.

With the view, therefore, of meeting just such a charge we evolved the present procedure of keeping records of the particulars of all patients treated in the various Government Centres.

Appendix I gives the form which enables a complete register of cases undergoing anti-rabic treatment to be kept at each treatment centre by the medical officer in charge. Immediately a patient has completed his course, the original form is submitted to the office of the Central Institute for filing, while the duplicate is retained at the treatment centre concerned. Three months after the last day of completion of treatment, a final report on the patient is submitted to the Central Institute by the responsible medical officer and this report certifies to the patient being in good health (or otherwise) on that date. This procedure, we consider, allows of the ready compilation of exact statistics.

IV. SUMMARY

1. Carbolyzed Anti-rabic Vaccine is an efficient and safe treatment for persons bitten by rabid animals.

2. It can be manufactured without additional staff in any well-equipped laboratory.

3. It can be distributed to any number of treatment centres where as good results attend its use as at the place of its production.

4. Better results have followed the employment here of a dosage of 5 c.c. daily of a 1 per cent. emulsion, over fourteen days, than of 2 c.c. of the same emulsion over the same period.

5. Carbolyzed vaccine is most practicable in the curative and prophylactic treatment of farm animals.

It can be easily administered in rural districts by veterinary officers.

6. It is a great advance on older methods of treatment in the wideness of applicability and economy of production of the vaccine. It is at once the most economical and utilitarian mode of treatment.

7. Bitten persons can be treated at or near their own homes, and thus—the all-important consideration of immediate treatment aside—they are spared the expenses connected with travel to, and residence in, a strange town.

ACKNOWLEDGMENTS

Our thanks are due to the Director of Health, Col. G. W. Heron, D.S.O., O.B.E., for his unfailing encouragement and for his affording us facilities which rendered the whole scheme of decentralisation possible. We are indebted also to Dr. Miftah, Director of the Pasteur Institute, Cairo, for many favours, but especially for his ungrudging assistance in the training of our personnel.

For the sake of completeness, we have considered it advisable to add three appendices :—

I. The form employed to register cases undergoing anti-rabic treatment.

II. Regulations for the control of rabies (in which is incorporated the procedure to be adopted in the case of the bitten animal). These regulations made under Art. 43 of the Ottoman Law concerning Diseases of Animals, and drawn up by Col. E. R. Sawyer, Director of Agriculture, have been in force since December, 1924.

III. Further measures adopted to free Palestine from rabies and hydrophobia.

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APPENDIX I

Local Form O. M. 186

Department of Health

REGISTER OF CASES UNDERGOING ANTI-RABIC TREATMENT.

No. Date District

Information about patient :

Name Age Profession
 Residence and Address
 Nationality Sent by
 Date of bite Animal inflicting bite
 Station where bitten

Wounds

| | Number | Gravity | Bitten on naked skin or through Clothing |
|-----------------|--------|---------|--|
| Head and neck | | | |
| Upper extremity | | | |
| Lower extremity | | | |
| Body | | | |

Have wounds been previously treated ? (cauterized) and when ?

Information about the animal.

Owner of animal Address
 What has become of the animal ?
 Other persons bitten, with names and addresses
 Other information (e.g. " dog bit unprovoked ")

Diagnosis.

1. Condition of animal from enquiry
2. Microscopical Researches (Negri bodies)
3. Experimental inoculation ? Result Date

Treatment.

1. When started
2. Vaccine and Dosage
3. Serial No. of Vaccine

| | | | | | | | | | | | | | |
|----------------|-------|--|--|--|--|--|--|--|--|--|--|--|--|
| 4. Attendances | Month | | | | | | | | | | | | |
| | Dates | | | | | | | | | | | | |

5. Conduct of patient during treatment
6. Accidents, if any, during treatment
7. Other remarks

Signature of M. O.

Final Report on Patient.

No. being three months after the last day of the completion of treatment,
 the patient is alive in good health (or otherwise) and is living at (address)
 District Signature of M. O.
 Date

APPENDIX II

REGULATIONS FOR THE CONTROL OF RABIES.

1. Every person having had in his possession or under his charge an animal affected with or suspected of Rabies shall give notice of the fact with all practicable speed to the Mukhtar, President of the Municipality or Police as the case may be. Failure to give such notice renders the person liable to a fine of £1 to £5 or to imprisonment not exceeding one month.

2. It is the duty of Mukhtars, Presidents of Municipalities and Police, on receiving such notice, to destroy the affected animal or to place it in strict isolation, and to transmit the information immediately to the District Governor or District Officer who will delegate the Veterinary Inspector to institute inquiries.

3. On confirmation of the disease and receipt of the report of the Veterinary Inspector, the District Governor or District Officer shall form a sanitary commission in accordance with the provisions of the said Ottoman Law, to execute the measures necessary for the control and suppression of the disease.

The commission shall be composed of the District Officer as president, and the Veterinary Officer, a representative of the Public Health Department, the Local Commandant of Police, and a member of the Municipal Council as members.

4. Every animal affected with Rabies shall be destroyed. Any animal whose behaviour leaves no doubt as to its being rabid shall be destroyed on the spot, and its body, in the case of dogs, cats and small animals, taken to the nearest District Veterinary Officer for disposal.

5. Animals bitten by rabid animals shall be dealt with as follows :—

(a) Donkeys, dogs, cats, monkeys, etc., shall be destroyed.

(b) Local camels; bulls, cows, calves, oxen, sheep and goats shall be slaughtered; but provided such animals are slaughtered within seven days of the date when first bitten, their carcasses, if free from other diseases, may be exposed for sale as food.

(c) Valuable horses, mules, bulls, cows and calves shall be destroyed, or
(1) strictly isolated for four months under the observation of Government Veterinary Officers and on premises approved by the Department of Agriculture, and (2) vaccinated with Anti-rabic Vaccine at the owner's risk and cost.

It is prohibited to sell such animals for any purposes whatsoever, during the period of observation.

6. Every animal bitten by a suspectedly rabid animal, and any dog which has been in contact with a suspectedly rabid dog shall either be destroyed or shall be strictly isolated :—

(a) Under the observation of Government Veterinary Officers, and

(b) In special cages, kennels, or stables, or on premises approved by the Department of Agriculture; and

(c) At the entire risk and expense of the owner; and

(d) For a period of six months in the case of dogs, or four months in the case of herbivorous animals or in both cases for such a period as will allow the diagnosis to be confirmed by the District Veterinary Inspector.

7. Every animal which has bitten a human being shall be placed in strict isolation and under observation for a period of at least ten days at the owner's risk and expense.

8. In no case may such animals be detained for purposes of observation by any private person or institution.

9. Animals will be destroyed by order of the District Officer or the District Veterinary Inspector, and no compensation will be paid in respect of such animals when they are :—

(a) Rabid or suspectedly rabid, or bitten by such animals ;

(b) In contact with a rabid or suspectedly rabid dog or other carnivorous animals.

10. The carcases of rabid or suspectedly rabid animals will be burned or deeply buried unskinned, but only after the examination by a District Veterinary Inspector or on the authority of the District Officer, in places selected by them.

11. The District Governor or President of a Sanitary Commission formed under the Law, shall in any locality where a case of rabies has occurred, issue a notice proclaiming the measures to be taken to control and suppress the disease, and the owners or persons in charge of animals shall observe and comply with such regulations.

12. The Administrative Authority, after notifying the public in the town or area, may proceed at any time to poison or destroy in any manner vagrant, stray, ownerless or collarless dogs and dogs not carrying the municipal tally.

13. Any person who fails to comply with any of the foregoing regulations or orders issued by the District Governor or Sanitary Commission for the Suppression of Rabies, or who does not assist in execution of such orders, shall be liable to prosecution before a magistrate under Art. 39 of the Ottoman Diseases of Animals Law of the 5th December, 1910 (1329 Moslem year), and on conviction to imprisonment not exceeding three months or to a fine not exceeding £20.

APPENDIX III

Apart from the preparation of a suitable vaccine for the treatment of persons bitten by rabid animals further measures were adopted to deal with the menace.

(a) The extermination of jackals and stray dogs—the common transmitters of the disease to human beings ;

(b) The education of the public in town, district and village regarding the nature of the disease, its method of transmission and the action to be taken by an individual who has been exposed to risk of infection from being bitten by a dog or other animal.

In regard to (a), the following action was taken by the Departments of Agriculture, Police and Prisons, and Health acting together :—

(1) The organisation of a constant campaign conducted by the Gendarmerie in country districts to lay bait poisoned with strychnine capsules in places frequented by jackals and pariah dogs. The campaign is carried out

along lines carefully worked out by the Department of Agriculture and during the present year has resulted in the finding of over 1,000 dogs and 750 jackals. The number of animals killed is known to exceed those actually found dead, as many which have swallowed poisoned baits travel some distance before the poison takes effect ;

- (2) In town areas the first step towards reducing the numbers of ownerless and pariah dogs was to regulate the registration and licensing of dogs kept by householders as guards or domestic pets. This registration is effected by the staff of the Municipalities under model regulations drafted by the government Departments concerned and adopted by all towns. Municipal employees are authorised to apprehend and destroy all dogs found at large not bearing on their collars a numbered tally indicating that they have been duly licensed.

When the number of ownerless dogs is found to be increasing in any given area of a town in spite of these measures, the assistance of the Police is called upon and the animals are destroyed by shooting.

From 1st January, 1923, until 30th June, 1925, over 20,000 ownerless dogs were destroyed.

Municipalities furthermore are required to make arrangements for the safe custody of dogs whose observation by a veterinary officer is necessary on account of their suspicious behaviour or unwarranted attacks on human beings. This is effected by the Municipalities providing 'Observation Kennels' constructed in accordance with plans prepared by the Department of Health and approved by the Chief Veterinary Officer.

In regard to (b), articles have been written for publication in the newspapers of the country drawing attention to the dangers of hydrophobia and informing the public where to present themselves for anti-rabic treatment if they chance to have been bitten by a dog or other animal. Pamphlets have been written in all the official languages for distribution in schools. Through the medium of the Department of Education these pamphlets reach a very large number of homes throughout the country and opportunity is taken by the teachers when giving these papers to the children to explain to them in short and simple language the essential facts concerning the disease and its prevention.

This educative work is of great value, for in hydrophobia no less than in other communicable disease the intelligent co-operation of the public is essential to secure the success of preventive measures undertaken by the Government.

ERYTHROCYTOSIS IN ARTIFICIALLY-INOCULATED MALARIA

BY

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AND

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(*Received for publication 12 September, 1925*)

In eleven cases of general paralysis inoculated subcutaneously with benign tertian malarial blood, Pijper and Russell (1925) found that an erythrocytosis occurred before the anaemia developed. In the graph showing the mean of the daily observations on these cases, the increase of the red cells occurs before the onset of the fever and is to the extent of about 750,000 cells per c.mm. In the charts of two cases, however, the erythrocytosis is shown to persist until the eleventh and twelfth days of the fever. The greatest increase recorded by these observers was 2,000,000 cells per c.mm.

On the other hand, R. M. Gordon (1925) found no increase of the red cells in three general paralytics inoculated with benign tertian malaria by means of anopheline mosquitos. Ben-Harel (1923) found in a series of 23 blood inoculations of *Proteosoma praecox*, in canaries, that the red cells diminished in numbers before the parasites were found in the blood stream.

The observations described in this paper were made upon cases of general paralysis inoculated with benign tertian malaria at Claybury Mental Hospital. Case No. 1 was inoculated by means of anopheline mosquitos by Lieut.-Col. S. P. James. The other cases were inoculated with defibrinated malarial blood by means of the method described by one of us elsewhere (1925). Cases Nos. 1 and 2 were females, 62 and 42 years of age; cases Nos. 3 and 4 were males, 40 and 50 years of age.

The red cell estimations were made with the Thoma-Zeiss counting apparatus and the haemoglobin estimations by means of Oliver's haemoglobinometer. The blood was collected between 9.30 a.m. and 10 a.m. every day from cases Nos. 1 and 2, and at 5.30 p.m. from

cases Nos. 3 and 4. Three red cell counts were performed in cases Nos. 3 and 4, the averages being taken as the correct readings. In cases Nos. 1 and 2 the red cell count was invariably repeated several times if there was much variation from the previous day's count. The average was taken of the two counts which approached each other most nearly. These methods of counting the red cells were used on account of the great error there is in the usual red cell count, Gordon (1925) placing it at 500,000 cells per c.mm. The temperature of each patient was recorded every four hours unless it was above normal; it was then recorded each hour until normal was regained. No drugs, other than those mentioned on the charts, were given.

Red Cells. The results obtained from the red cell counts are shown on the charts. All four cases show very clearly an erythrocytosis preceding the anaemia. The relation of this increase of the red cells to the onset of the fever was very variable. In case No. 1 it occurred before and during the onset, in case No. 2 it coincided with the onset, in case No. 3 the greatest increase occurred after the fifth rise of temperature and in case No. 4 after the eighth. As the count was not commenced until six days after the onset of the fever in this case, it is possible that there had been an erythrocytosis previous to the one recorded.

This erythrocytosis is in agreement with the findings of Pijper and Russell, but not with those of R. M. Gordon or Ben-Harel. As neither of the two latter workers record observations made every day, it is possible that an erythrocytosis occurred between the observations. Cases Nos. 1 and 2 of the present series show that the erythrocytosis may persist for only a few days. Gordon does, however, describe one case on whom red cell counts were performed every day, but the estimations were not commenced until the ninth day of infection. There were then 6,000,000 red cells per c.mm. As this is a comparatively high figure for an untreated general paralytic in England, perhaps the count represents an erythrocytosis. In case No. 1 of the present series, 6,000,000 red cells per c.mm. were found on the tenth day of infection.

The duration of the anaemia is of interest. In cases Nos. 1 and 3 it persisted for at least 11 and 13 days after the commencement of the quinine. In case No. 4 it persisted for at least six days after the course of quinine had been started, becoming more

profound although no further febrile paroxysms had occurred. This case corresponds with one described by Gordon. In this patient, a female aged 13 years, the red cells continued to fall although the parasites had disappeared from the peripheral blood. In case No. 4 of the present series the same condition occurred. The lowest red cell count found in the present series was 1,300,000 cells per c.mm.

Recovery from the anaemia took place in a comparatively short time. In cases Nos. 1, 2 and 3, the red cells reached 5,000,000 per c.mm. in about three weeks. During this period the cells increased by 1,700,000 per c.mm. in case No. 1, by 2,600,000 per c.mm. in case No. 3, and by 3,300,000 per c.mm. in case No. 2. In these cases the degree of the anaemia did not influence the time required for normal to be regained. James (1920) gives a chart, after Ziemann, showing regeneration of the red cells after naturally-acquired malaria. In this case the normal was regained 16 days after the anaemia. Case No. 4 shows a more rapid recovery, 5,000,000 red cells per c.mm. being reached in about a week after the anaemia. In cases Nos. 1 and 2, both of whom received neokharsivan in addition to quinine, the regeneration of the red cells was no more rapid than in case No. 3, and less rapid than in case No. 4. Neither of the two latter cases were given this preparation. As neosalvarsan has a definite parasitocidal action on *Plasmodium vivax*, both in the naturally-acquired infection (D'Esterre, 1920; Nieuwenhuyse, 1921; Johnson, Gilchrist and Hay-Michel, 1921) and in the artificially-inoculated form (Pijper and Russell, 1924), it might be expected that the parasites would be destroyed more rapidly in cases Nos. 1 and 2, than in cases Nos. 3 and 4 and, consequently, the red cells would be regenerated more rapidly. This did not occur.

In cases Nos. 3 and 4, red cell counts were continued after the normal had been regained. In each case an erythrocytosis was found. The highest count obtained in case No. 3 was 5,920,000 cells per c.mm., and in case No. 4, 6,280,000 per c.mm. This post-anaemia increase of red cells has been observed, according to Ben-Harel, in the naturally-acquired infection, and this worker noted it in canaries who had suffered from infection with *Proteosoma praecox*. In two of the birds the erythrocytosis persisted for a little over six

months. Of the two cases under review, No. 3 showed a count of 4,990,000 cells per c.mm., with a haemoglobin percentage of 65 on July 10th, nearly six months after the last rigor, while No. 4 gave a count of 5,870,000 cells per c.mm. and haemoglobin at 90 per cent. on July 22nd, exactly six months after the last rigor. Although the highest point reached in these two cases was not permanent, in neither case was the count lower than it had been before the malarial anaemia. In case No. 4 it was about one million higher.

Haemoglobin and Colour-Index. In cases Nos. 3 and 4 estimations were made of the haemoglobin. The results, with the colour-indices, are shown on the charts. The haemoglobin does not vary to the same extent as do the red cells and the colour-index remains low. The colour-index in case No. 4 is of the secondary anaemia type throughout. This corresponds with the two cases reported by Gordon. This agreement is not seen in case No. 3, for the index exceeds unity on two occasions in this patient. In both cases it falls very low at certain periods.

Number of Parasites. In cases Nos. 1 and 2 the relative number of parasites was found by counting the total number of asexual forms in 100 fields of the microscope, using 1/12th in. oil-immersion objective and thin blood-films. Although the actual number of asexual parasites was very different in the two cases, both patients show that there is a tendency for the temperature to vary with the number of parasites in each patient. The temperatures of the two cases were recorded every hour when above normal, at other periods every four hours. In the cases investigated by Pijper and Russell the temperatures were recorded every four hours throughout. In their cases there is no clear relationship between the number of parasites and the degree of fever.

Previous Malaria. Three of Pijper and Russell's nine cases were known to have had previous attacks of malaria, whilst the remainder had a doubtful history with regard to this point. Of the present series, cases Nos. 1 and 2 are known not to have suffered from the infection previously. It is clear, therefore, that the increase of the red cells preceding the anaemia does not necessarily bear any relation to previous malaria, for the increase was found both in patients who had suffered from previous malaria and in those who had not.

Sex. As the increase of the red cells preceding the anaemia was found in both female (Nos. 1 and 2) and male (Nos. 3 and 4) cases, the erythrocytosis is not dependent upon the sex of the patient.

Mode of Inoculation. Case No. 1 was inoculated by means of anopheline mosquitos, the remaining patients by means of subcutaneous injection of malarial blood. Pijper and Russell's cases were inoculated by the latter method. The pre-anaemia erythrocytosis was found in all the cases. The increase of the red cells is not dependent upon the method of inoculation, therefore, so far as these two methods are concerned.

SUMMARY

(1) In four cases of general paralysis inoculated with benign tertian malaria an erythrocytosis was found to precede the anaemia. The erythrocytosis occurred whether the inoculation was performed by means of mosquitos or by subcutaneous injection of malarial blood. It was independent of the sex of the patient and of a history of previous malaria. The succeeding anaemia occurred, or persisted for several days after the cessation of the fever. Regeneration of the red cells was complete within three weeks, although the degree of anaemia was very different in the four cases. An erythrocytosis was found to follow the anaemia.

(2) The haemoglobin was found, in two cases, to vary with the red cells, but regeneration was less rapid. The colour-index was, as a rule, of the secondary anaemia type. At certain periods it became as low as .5.

(3) In two cases the number of parasites was found to vary approximately with the degree of fever, although the number of parasites was very different in the two cases.

Our thanks are due to Dr. G. F. Barham for permission to publish the above observations from the records of Claybury Mental Hospital, and to Dr. F. Paine for his kind assistance.

We are again indebted to Lieut.-Col. S. P. James for his kind advice.


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EXPLANATION OF CHARTS

- A. Number of red cells in millions per c.mm.
- B. Temperature in degrees, Fahrenheit.
- C. Number of parasites in 100 fields.
- D. Haemoglobin percentage.
- E. Colour-Index.
- I. Day of Inoculation.

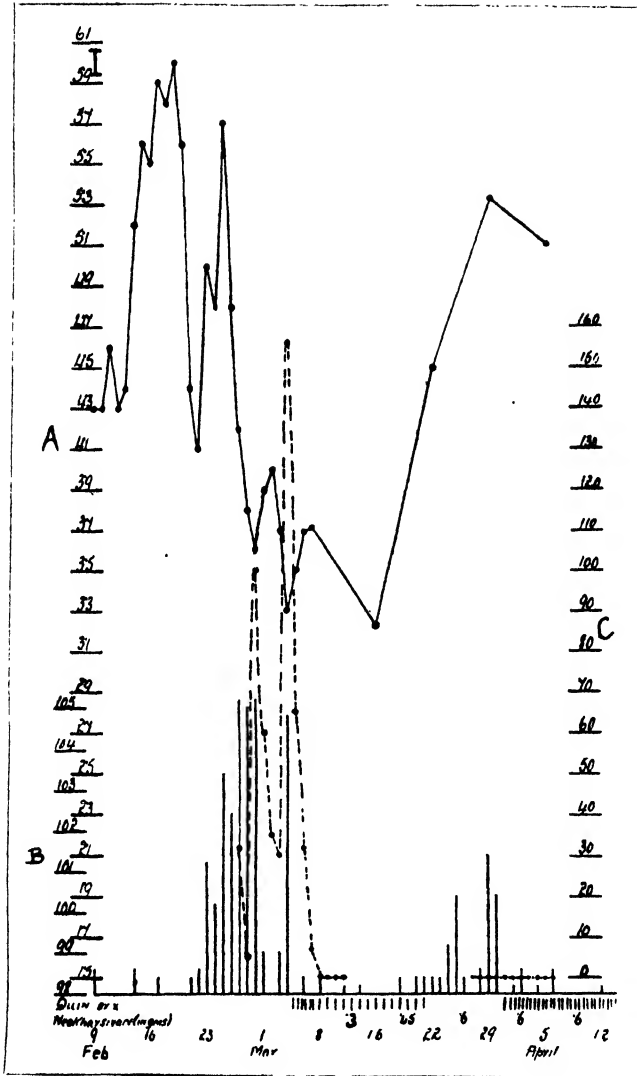
Red cells — — —

Parasites — — — — and 

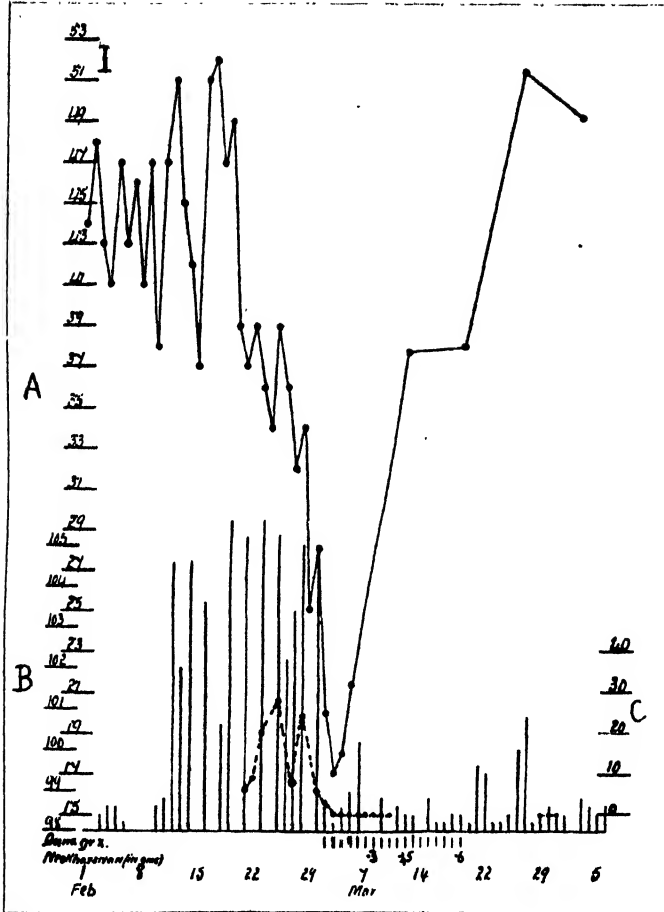
Haemoglobin — — — — —

Colour-index

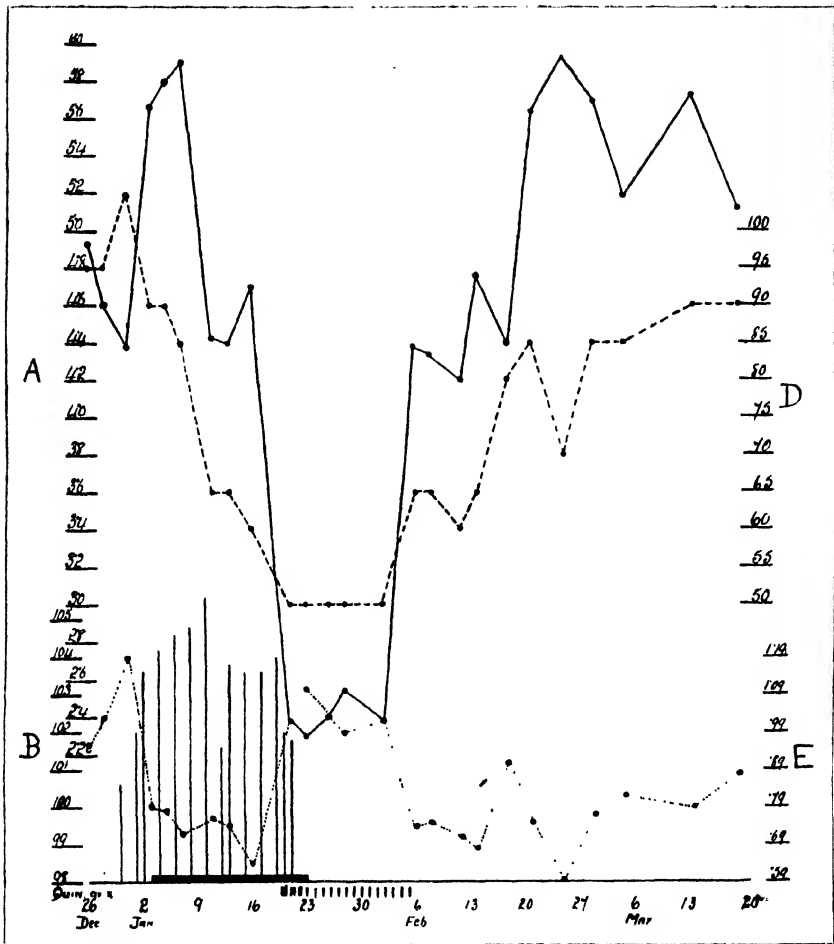

Temperature |

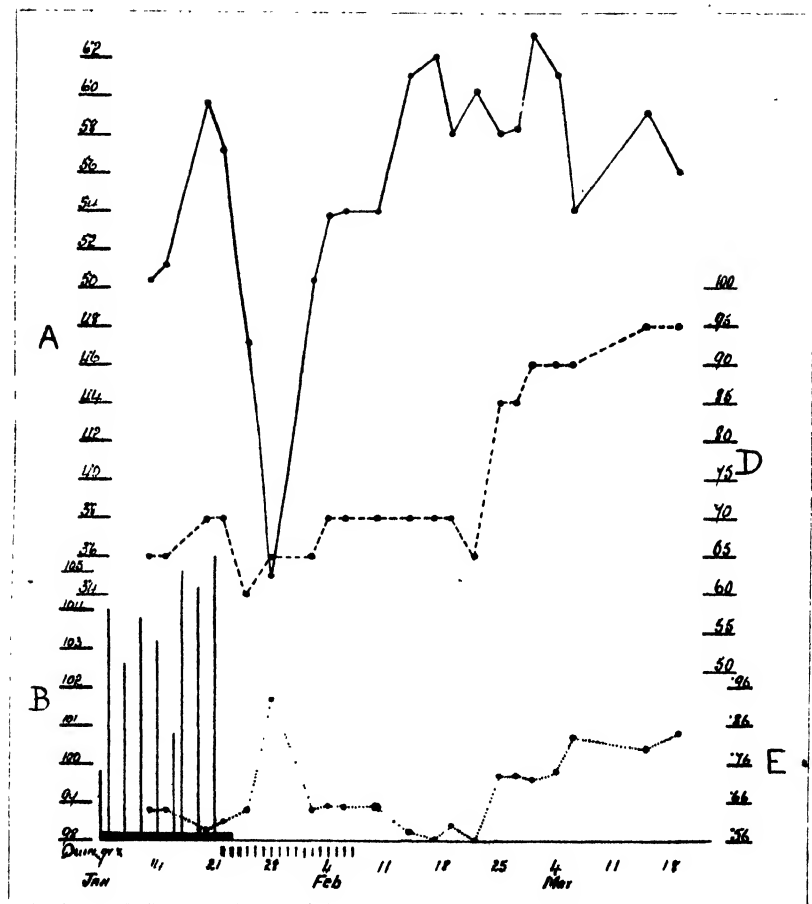
CASE NO. 1



CASE No. 2



CASE No. 3



CASE No. 4

THE EFFECT OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE HEALTH OF THE WEST AFRICAN NATIVE

BY

R. M. GORDON

(From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone)

(Received for publication 28 October, 1925)

PLATE VII

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I. INTRODUCTION

The limitations of the work must first of all be made clear.

1. It deals solely with the West African male native, as studied at Freetown, and any conclusions drawn apply only to this race.

2. It is concerned only with the effects of the infection on the individual; it is not concerned with the importance of the infected individual as a propagator of the disease.

3. The lesions and symptoms produced by migrating larvae are considered to be a separate subject and are not discussed.

4. The effect of treatment is not considered; whereas this subject is obviously one of great importance, it appears to the writer that the first and most important consideration is the effect, if any, of ancylostome infection on different races of mankind.

5. No distinction is drawn between infection with *A. duodenale* and *N. americanus*. Darling and others (1920) and Darling (1922) show that *A. duodenale* is more important as a producer of anaemia than an equal infection with *N. americanus*. Adler (1925) gives the proportion of *A. duodenale* to *N. americanus* in Freetown as 1 : 10.

Malaria and ankylostomiasis are usually accepted as the most important diseases affecting natives in West Africa, and certainly entail a greater expenditure of money than any other two diseases. The effects of Malaria are definite, and the pathological changes which it produces in the individual can be demonstrated and classified, while the value of its eradication is too obvious to need argument. The position as regards ankylostomiasis appears to be entirely different; the causal organism and its life cycle are established, and the value of its eradication appears generally accepted, but the effect of the worm on its host and the pathological lesions produced by it seem to be subjects evoking the widest differences of opinion, ranging from those who regard ankylostomiasis in general as having little effect on the human host, to the other extreme, which considers that any infection, however light, is responsible for illness of the individual, and calls for immediate treatment; between these two extremes are to be found numerous observers who consider that a certain concentration of worms must be present in the gut before any symptoms appear. Unless it is conceded therefore, that any infection, however small, is pathogenic, it is obvious that any attempt to define the pathogenicity of ankylostomiasis must include a statement showing the degree of infection of the individuals considered; it is doubtful if any value can be attached to mere comparisons of infected and uninfected individuals, such comparisons being frequently made and often illustrated with photographs showing poorly developed individuals suffering from ancylostome infection as diagnosed by the finding of ova (the number not being stated) in the stool, and well-developed, athletic-looking individuals free from infection. The two photographs accompanying the present article represent, in the one case, six boys selected at random from amongst the heaviest infections, and in the other case, six boys selected at random from amongst the negative or lightly infected group; if anything, it is the heavily infected group which appears to show the best physique. In the enormous bibliography of hook-worm

disease definite figures of the degree of infection of the cases considered are surprisingly few, this lack of figures being, of course, largely due to the fact that until the publication of Stoll's (1923) method of estimating the number of ova in a given sample of faeces, there was little uniformity of opinion as to what constituted a 'light' or 'heavy' infection, and it is probably this lack of uniformity of opinion that has led to the surprising diversity of statements concerning the pathogenicity of ankylostomiasis.

Another difficulty encountered is that the literature dealing with ankylostomiasis appears almost entirely to ignore concomitant infections with other gut helminths, even when the pathogenicity of such helminths is admitted by the authors of the publication.

During the first part of the present work, which consisted in examining prison cases, the writer was impressed with the high proportion of Ancylostome cases which were also infected with Ascaris or Trichuris or both; in subsequent examinations, therefore, a count was kept of these ova, and a classification made of the cases on the same lines as in the ancylostome work. Infection with the larvae of *Strongyloides stercoralis* was also common in Freetown, as noted by Maplestone (1924); they are not recorded here owing to the difficulty of estimating the degree of infection, which varied enormously with the consistency of the stool. Ova of a cestode (probably *T. saginata*) and on one occasion those of *S. mansoni* were also noted; both infections were rare and when seen the ova were too few in number to be worth estimating.

Granted that ankylostomiasis is pathogenic, there still remain great differences of opinion as to the manner in which this pathogenicity manifests itself in the individual and what lesions, if any, the worm produces in the gut. Thus a perusal of the 'Rockefeller Bibliography of Hookworm Disease' (1922) shows that it tabulates articles on almost every conceivable sign and symptom ranging from arthritis to night-blindness. The present writer attempted to compile a table summarising the views of modern authorities on the subject, but it was found that such a table became hopelessly unwieldy, as it had to include columns for almost every system and organ of the body. Most, though not all, authorities are, however, agreed that *ancylostome infection adversely affects the host by producing* (1) *Anaemia*, (2) *Poor physique*, (3) *Mental dullness*, (4) *Lack of*

energy. It is with these four points that the present paper, which deals with 137 natives, of whom 114 (83 per cent.) were infected, is concerned.

The number of cases dealt with is small and it may at first seem unnecessary to add them to the already overburdened literature on the pathogenicity of ankylostomiasis, but the information concerning these cases has been made as comprehensive and as exact as possible, whereas, as already stated, by far the greater proportion of published literature on this subject lacks figures showing the degree of infection of the cases under consideration and abounds in statements associating this or that symptom with ancylostome infection, such statements being unsupported by any proof except the finding of an unestimated number of ancylostome ova in the stool of the patient. It is interesting in this connection to consider the following statements. Stephens (1916), quoting Löbker and Bruns (1906), writes 'Whilst up to modern times it has been generally maintained that the great majority of worm diseases cause more or less marked symptoms, the exact investigations of the last few years have made it plain that the great majority of people with worms are not only perfectly healthy, but the most careful clinical observations show no single sign of any ill-effect of the intestinal parasites on the health of the host (Löbker and Bruns).' Clayton Lane (1917) points out that the reference date of Löbker and Bruns is 1906 and dismisses the whole statement as being out of date ; referring to the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease and the Rockefeller International Health Commission, Lane continues as follows : ' It is obvious that the opinions based on this enormous experience, which in the five years of the Sanitary Commission's existence, covered over 1,300,000 persons, carries a weight borne by those of no other person or scientific body in the world ; and that should any individual elect to differ, the onus of fully justifying his own attitude must lie with himself.' Such a statement as this has the natural effect of deterring the individual observer from adding his small quota to so vast an array of figures ; no one who has studied the literature of the Rockefeller Commission can but be impressed with the magnificent work published and the splendid results obtained by this body of investigators. Yet at the time of Lane's paper (1917) the present

writer is unaware of any paper published by the Rockefeller Commission which dealt with the degree of infection of the persons considered, except in a few instances where a rough comparison is made by the general appearance of the number of ova in the stool; the whole of the 1,300,000 cases referred to are only considered as a comparison of infected and uninfected individuals; thus Strong (1916) investigated the effects of ankylostomiasis on the physique and mentality of 115 school children and drew the following conclusions.

' (1) *Our figures show that hookworm disease interferes with physical development. Treatment alleviates this condition to a considerable extent. Apparently young children can regain most of the physical conditions, if not all, which they have lost due to the infection.* But the data do also very strongly suggest that the severer the infection and the longer it persists, the less likely it is that the child will ever reach his normal physical development.' He draws the following conclusions as a result of the mental tests. 'The figures show, then, that hookworm disease unmistakably affects mental development. *Treatment alleviates this condition to some extent but it does not, immediately, at least, permit the child to gain as he would if he had not had the disease. And the figures apparently further show that prolonged infection may produce prolonged effects upon mentality—effects from which the individual may never entirely recover.*' No estimation was made in these cases of the concentration of ova in the stools or the number of worms in the intestines of the children. The consideration of these cases, therefore, resolves itself essentially into a comparison of infected and uninfected cases.

Our present knowledge shows that such a comparison is liable to very wide error. To quote one instance only: Hill (1923) records 282 cases, of whom 142 with 1 to 2,099 ova per gm. showed no symptoms, while 57 with 2,100 to 5,099 only showed very slight and indefinite symptoms. A few months prior to the publication of this paper, Lane (1923b) wrote as follows: 'It is at least certain that there is a growing mass of evidence that the so-called carrier is improved in health and working power by disinfestation, and I know of no published evidence suggesting that there is any limit below which infestation is immaterial. Statements of personal belief on this matter appear misplaced. The fact seems to be that there is

no satisfactory evidence, either for or against the belief that the lightest infestations are immaterial to their host.' This statement would appear to the present writer to be a very fair summary of the state of affairs at the time the paper was published, except that the latter portion of the statement seems to negative the value of the remarks as regards the so-called carrier being improved in health and working power by disinfestation, and would also appear to indicate that Lane has considerably modified his previous views, as in 1919 he states : ' Each year adds to the accumulated facts indicating that even light infections are a definite handicap to growth in wisdom and stature, and to the full possession of that modicum of health and wealth which makes life worth living.'

A search of the literature has shown that the following are the more important papers dealing with the effects of ankylostomiasis on the host when the degree of infection is approximately known. (1) Darling, Barber and Hacker (1920) ; (2) Darling and Smillie (1921) ; (3) Smillie (1922) ; (4) Hill (1923) ; (5) Cort, Payne and Riley (1923) ; (6) Mhaskar and Kendrick (1923) ; (7) Cort (1924) ; (8) Mhaskar (1924) ; (9) Chandler (1925) ; (10) Stoll and Tseng (1925). The work of these writers on the pathogenicity of ankylostomiasis is almost entirely concerned with the question of anaemia, and there appears to be a great need of further investigation as to its effects on the health and mentality of different native races.

It will be noted that the above summary refers only to ankylostomiasis ; the writer is unaware of any publication dealing with the pathology of *Ascaris* or *Trichuris* infection based on a knowledge of the intensity of the infection in the individuals concerned.

II. CASES AVAILABLE, CLASSIFICATION OF CASES, COLLECTION OF MATERIAL

Only cases which were under constant supervision and discipline were selected. They were chosen from amongst three sections of the native male community in Freetown. (1) 49 youths aged 10 to 22 (average age 18) attending school, the majority as boarders ; (2) 40 City Police of all ages from 23 to 50 ; (3) 48 gaol prisoners of all ages from 17 to 49. One hundred and thirty-seven cases

were thus examined, the work occupying about four hours daily for a period of eight months.

In every case the examination, as regards physique, mentality and energy, was carried out by the officer in charge of the institution concerned, according to a fixed scheme previously carefully discussed and agreed upon between the officer and the Laboratory. In order to avoid any bias that might result from any previous knowledge, the officers in charge of the institutions did not know the degree of infection of the inmates, and the Laboratory was unaware of the classification of the cases it was examining. The haemoglobin percentage, as shown by a Talquist scale, was estimated by the writer who was not aware of the identity of the particular case he was at the time investigating. In addition to the haemoglobin estimation, each case was examined as regards three other categories : (1) Physique and general fitness, (2) Mentality, and (3) Energy, and placed in order of merit, in an *A*, *B* or *C* class in each of these three categories.

The physical examination requires no special comment ; it was not necessarily a medical examination (though the doctor's report was usually available) but consisted in placing the native in class *A*, *B*, or *C* according to his general physique and fitness when seen stripped.

The mental examination was not directed to ascertain how much the individual knew, but rather to discover his mental alertness and ability to learn ; thus a boy at the head of his class might be placed in category *C* because he had gained his position at the head of the class by remaining behind when brighter boys had been moved on. The mental classification was comparatively simple in the case of the boys and police who were being regularly taught and questioned, but in the case of the gaol prisoners, it had to consist of an examination of the man's mental ability as judged by the answers he gave to a series of simple questions.

Energy was defined as the keenness with which an individual attempted any mental or physical task allotted to him ; it was frequently found that this classification gave very different results from the other two ; thus a native might be classed as physically poor (*C*) and mentally dull (*C*), but as regards energy very good (*A*) because, though his ability to perform any task, whether physical

or mental, allotted to him, was bad, yet the energy he showed in trying to perform the task was excellent.

Strong (1916) has published a long and very carefully detailed account of his investigations on the effects of ankylostomiasis on the physique and mentality of 115 school children living in a 'hookworm infected county.' He divided the children into five groups according to whether (1) They were not infected (Group A); (2) They were infected but not treated (Group B); (3) They were infected and later cured (Group C); (4) They were infected and treated but not completely cured (Group D); or (5) They were infected and treated but the final condition of their infection could not be determined (Group E).'

The tests applied to these groups were extremely ingenious and interesting, but appeared too complex for use in a native community such as Freetown. The question of Strong's conclusions from these tests has already been dealt with (see Introduction).

The classification having been completed, the case was issued with a faeces container marked with his number and, as a rule, the specimen was passed under the observation of an individual appointed for the purpose; the specimen was then dispatched to the Laboratory and examined according to the technique described later. The information regarding the case was therefore tabulated on two forms, one form being filled in by the Laboratory and the other by the person in charge of the school, barracks, or gaol, the two portions being compared together only when the work was completed. In the case of the 48 gaol prisoners only ancylostome ova were counted and facilities for haemoglobin estimation were not available; in the case of the 40 city police and the 49 youths attending school, *Ascaris* and *Trichuris* ova were counted in every case, and in 84 of the 89 cases the haemoglobin percentage was also estimated.

III. TECHNIQUE OF ESTIMATING THE NUMBER OF OVA IN THE FAECES

Stoll (1923) published a technique for counting hookworm eggs in faeces, and in 1924, he published a further paper in the conclusions of which he states 'A relationship of approximately 1 : 2 : 4 is found to exist in general between the weighed amounts of formed, mushy (unformed), and diarrhoea faeces, passed per day. This affords an

easy interpolation by which to bring counts made on faeces of any of the three categories to a similar plane, the basis of formed faeces, so that they can be compared *inter se*.' The reader is referred to these two papers for details of the technique, which was exactly adhered to except for the following trifling modifications and additions. (a) Stoll balances the container and its faeces on the scales and removes 3 gm. into a large test-tube containing three glass beads and 45 c.c. of $\frac{N}{10}$ NaOH. The writer found it simpler and less messy to stir thoroughly the specimen of faeces and weigh out 3 gm. into a small metal container previously balanced on the scales, and then slide the metal dish, containing the 3 gm. of faeces, into the large test-tube and add 45 c.c. of $\frac{N}{10}$ NaOH and three glass beads. The metal dish aids greatly in rapid emulsification of the faeces when shaking the tube, and is also very convenient for dealing with liquid faeces. The dish referred to is made of the thin tin used in sealing boxes of cigarettes sent to the tropics; the tin should be cut into a square and the four corners bent so as to form a rectangular water-tight trough measuring about 2 in. \times $\frac{3}{4}$ in. \times $\frac{1}{2}$ in. (b) Stoll says the diluted faeces 'was immediately sampled with a pipette graduated at 0.15 c.c.' It will be found in practice that in some cases faecal debris adheres to the outside of the pipette and interferes with accuracy by draining into the fluid which is being discharged on to the counting glass; in order to avoid this error it is advisable to draw up fluid past the 0.15 c.c. mark, rapidly wipe the outside of the pipette with a wisp of wool, discard the excess of fluid and discharge the remaining 0.15 c.c. on to the counting glass. It is necessary to perform this operation very rapidly in order to avoid sedimentation occurring. (c) Stoll measures 0.15 c.c. of the diluted faeces on to a large slide and covers this with a single 22 \times 40 mm. coverslip. The writer found it more convenient to use three amounts of 0.05 c.c. and count each separately. (d) A small point, but one which, if neglected, interferes with accuracy, is that the surplus uncovered fluid lying along the edge of the coverslip should be first examined, otherwise the rapid drying up which occurs in the tropics will render the counting of ova difficult. (e) At the commencement of the work it was found that certain bodies, probably derived from some

vegetable in the native dietary, imitated unfertilised *Ascaris* ova with such extreme fidelity, both as regards size and morphology, that they necessitated careful examination with the high power in order to differentiate them from ova ; it was therefore decided to include only ' fertile ' *Ascaris*, *Ancylostome* or *Trichuris* ova in the counts. (f) Chandler (1925) advocates examining uncovered preparations, as by this method one can blow aside obscuring flocculi of undissolved faecal debris, while doubtful egg-like objects can be verified by blowing on the fluid and causing them to roll about. The writer tried this method prior to the publication of Chandler's paper and abandoned it because it was found that any current of air occurring in the laboratory caused the ova in the fluid to move about and lose their position in the field which was being counted.

IV. ACCURACY OF STOLL'S METHOD AND ITS VALUE IN COMPARING THE DEGREE OF INFECTION IN DIFFERENT INDIVIDUALS

Stoll (1923), when describing his method of estimating the number of hookworm eggs in faeces, claimed that this technique was ' accurate to within 10 per cent.', while Maplestone (1924), who tested the accuracy of the method by control counts of ova made with saturated salt solution, and cultures of larvae made from the same faeces sample, came to the conclusion that the method was ' not accurate to within 10 per cent.'

In order to compare together the ovum content of stools of different consistencies, Stoll (1924) advocates the taking of a formed stool as a standard and multiplying the ovum content of a mushy stool by two and a liquid stool by four (this being the method adopted in the present work) ; Chandler (1925) regards mushy stools as normal for the Indian native, and therefore divides the results obtained from formed stools by two and multiplies those of liquid stools by two ; whatever the accuracy, therefore, of any technique for estimating the number of ova in a single given sample of faeces, it is obviously absurd to discuss the finer points of accuracy of such a technique when applied to the estimation of the average number of ova in a series of stools varying in consistency, for the definitions ' formed ' ' mushy ' (or ' semi-solid ') and ' liquid ' are not fixed

definitions, and an examination of even as few as fifty specimens will convince any worker on this subject that every variation between these standards is to be found, where one observer will define a stool as mushy and multiply his result by two, another will call it liquid and multiply his result by four. A clear-cut distinction must, therefore, be drawn between estimating the number of ova in a given sample of faeces, and the comparing together of the average number of ova passed by different individuals on different occasions; it is obvious that the first can be performed to any degree of accuracy if sufficient time and care are expended; thus Stoll's method admittedly does not detect the presence of ova in the stool if less than 100 per gm. be present. Therefore all single worm infections will be missed. Now Lane's (1923-1925) method will detect less than this concentration and clearly, therefore, his method is more accurate and, therefore, more suitable for such an estimation. The present work, however, is not concerned with such an estimation, but is concerned with the comparison of the ovum content of the stools of different individuals on different occasions; now the ovum content of such stools may vary according to the quantity of faeces passed (presumably the less food taken the smaller the quantity of faeces and the greater the concentration of the ova), the consistency of the faeces, and the fecundity of the worms. When such a number of uncontrolled factors exist the more minute points of accuracy are of little importance; what is required is a method whereby negative (by negative is meant less than 100 ova per gm.), moderate, and heavy infections can be compared together, and for this purpose Stoll's technique seems well adapted. That such comparative accuracy is obtainable by Stoll's method appears to be proved by the figures given in Table I. It is of interest to note that the counts of *Ascaris* and *Trichuris* infections do not correspond nearly as closely as do those of *anclostome* infections, a possible explanation being that fewer worms are present in the two former infections and that the variations in the fecundity of the worms are, therefore, more clearly noticeable.

For further particulars regarding the comparative accuracy of Stoll's (1923) and Clayton Lane's (1923-1925) method, the reader is referred to articles by Sweet (1924) and Chandler (1925b).

Showing the number of ova actually counted in 0.01 gm. of faeces in the same individual on three occasions, amongst a series of 114 positive Ancylostome cases, 16 positive Ascaris cases, and 39 positive Trichuris cases. Solid specimens are shown by black figures. When a specimen was 'mushy' or 'semi-solid' the result has been $\times 2$, and when liquid $\times 4$. For the purpose of reference the numbers given to the cases have been adhered to throughout the tables and text.

NOTE.—The occurrence of a decimal point in a few of the cases is due to the figure being the average of a series of counts on the same specimen.

TABLE IV.—Sixteen Positive Ascaris Cases.

[illegible]

V. EFFECTS OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE (A) HAEMOGLOBIN PERCENTAGE, (B) PHYSIQUE AND GENERAL FITNESS, (C) MENTALITY, (D) ENERGY, AND (E) URINE, OF WEST AFRICAN NATIVES

In the results which follow, the degree of infection is expressed as the average number of ova per gm. of faeces, this figure being the mean of three Stoll counts on each individual case, except that five cases amongst the 137 examined for ancylostome infection, and two each amongst the eighty-nine *Ascaris* and eighty-nine *Trichuris* series were only examined on two occasions, the natives concerned having left the Institution before the third examination was completed. The counts were usually made at intervals of four to seven days, but occasionally longer periods intervened. The expression 'average number of ova per gm. of faeces,' when applied to a number of cases constituting a group or class, includes negative cases, that is to say, it is the figure arrived at by adding together the average number of ova per gm. of faeces of each member of the group and dividing the result by the total number of individuals in that group; the same rule holds good for the heading 'average number of ancylostomes per individual.' The figures for the number of ancylostomes are, of course, only roughly approximate, but they are included in the tables as they would appear to give a more concrete idea of the degree of infection; the estimation of the number of ancylostomes is based on the supposition that every forty ova per gm. of faeces represents one adult female worm; this relation between ova per gm. and parent worm is given by Stoll (1923b) as 44 to 1, by Darling (1922) as 22 to 1, by Lane (1923) as 33 to 1, and by Davis (1924) as 85 to 1. To this figure must be added the proportion of male worms which is here estimated as two males for every three females (see Darling, Barber and Hacker (1920), Stoll (1923b), Adler (1925)). The final formula is, therefore, $-\frac{x}{40} + \frac{2}{3} \left(\frac{x}{40} \right)$ where x is the number of ova per gm. of faeces. Figures of the number of *Ascaris* and *Trichuris* present in the gut are omitted, as there appears to be no authoritative statement as regards the average daily egg production of these two species, except those of Davis (1924) who, as the result of the examination, and subsequent treatment, of

sixteen positive *Ascaris* cases computes the average number of eggs per female, per gm. of faeces, to be 3,466, and Moosbrugger, as quoted by Brumpt (1922), who, as the result of an autopsy, gives the *Trichuris* figure as seven ova per female per gm. of faeces.

The influence of *Ancylostome*, *Ascaris* and *Trichuris* infections will be considered as regards their effects on five conditions. (A) Haemoglobin percentage. (B) General physique and fitness. (C) Mentality. (D) Energy. (E) Urine.

(A) *Haemoglobin percentage.* It will be seen that Table II lends no support to the commonly accepted view that *Ancylostome* infection tends to lower the haemoglobin reading; nor do *Ascaris* or *Trichuris* infections appear to have any influence, for it can be seen that the group with the higher haemoglobin reading actually contains a slightly higher average degree of infection than the group with the lower haemoglobin reading. It is perhaps unfortunate that the haemoglobin readings in the two groups approximate so closely, but this was unavoidable as the haemoglobin readings in all the West Africans examined fell between 90 and 70 per cent.

TABLE II.

Showing the percentage of individuals infected with *Ancylostome*, *Ascaris*, and *Trichuris*, and the average degree of infection, in each of two groups of West African natives classified according to the haemoglobin reading.

| Groups based on Haemoglobin per cent. | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of <i>Ancylostomes</i> per individual |
|---------------------------------------|--------------------------|--------------------------|----------------|------------------|---|----------------|------------------|---|----------------|------------------|---|
| | | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | |
| A 81-90 % Hb | 57 | 86 | 19 | 47 | 3,670 | 1,878 | 125 | 21,100 | 42,630 | 2,230 | 150 |
| B 71-80 % Hb | 25 | 84 | 16 | 36 | 2,890 | 1,947 | 118 | 23,100 | 19,500 | 933 | 120 |

The question now arises whether intense infection produces any marked change; with the object of investigating this point the ten heaviest infections in the case of each worm are set forth in Table III.

TABLE III.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the haemoglobin reading for each case.

| ANCYLOSTOME | | | | ASCARIS | | | TRICHURIS | | |
|-------------|----------------------|---------------------------------|---------------------------------|---------|-----------------------|---------------------------------|-----------|-----------------------|---------------------------------|
| Case | Haemoglobin per cent | Number of ova per gm. of faeces | Computed number of Ancylostomes | Case | Haemoglobin per cent. | Number of ova per gm. of faeces | Case | Haemoglobin per cent. | Number of ova per gm. of faeces |
| 44 | 70 | 23,100 | 962 | 38 | 80 | 42,630 | 20 | 80 | 2,230 |
| 71 | 80 | 21,100 | 879 | 74 | 75 | 19,500 | 15 | 80 | 1,000 |
| 33 | 80 | 15,600 | 650 | 66 | 75 | 17,733 | 73 | 75 | 933 |
| 35 | 85 | 13,700 | 570 | 30 | 85 | 17,400 | 74 | 75 | 900 |
| 60 | 90 | 13,600 | 567 | 115 | 80 | 10,300 | 60 | 90 | 460 |
| 76 | 85 | 13,400 | 558 | 117 | 80 | 9,430 | 119 | 75 | 400 |
| 38 | 80 | 13,200 | 550 | 57 | 85 | 9,130 | 14 | 80 | 360 |
| 47 | 70 | 13,100 | 546 | 72 | 75 | 8,700 | 49 | 90 | 330 |
| 54 | 90 | 12,660 | 527 | 116 | 80 | 4,260 | 22 | 85 | 300 |
| 55 | 70 | 11,200 | 466 | 65 | 85 | 4,160 | 124 | 85 | 266 |

It will be seen from Table III that, broadly speaking, two-thirds of the heaviest infections in the case of each worm fall into the higher haemoglobin group; moreover, if we consider the haemoglobin content of those natives who were uninfected, we find that of the eighty-two cases in which haemoglobin readings were made, twelve were negative as regards ancylostomes, and of these eight fell into the higher haemoglobin group and four into the lower. Sixty-seven were negative as regards Ascaris, and of these forty-six fell into the higher haemoglobin group and twenty-one into the lower. Forty-six were negative as regards Trichuris, and of these thirty fell into the higher haemoglobin group and sixteen into the lower. Thus it appears that roughly two-thirds of the heaviest infections and two-thirds of the negative cases fell into the higher haemoglobin group, and this figure corresponds with the relative size of high and low haemoglobin groups amongst the total eighty-two natives examined, that is, 57 to 25.

From these facts it seems clear that there is no correlation between intensity of infection in the individual and the haemoglobin reading. It might, of course, be argued that all the natives under consideration exhibited some degree of anaemia; this may be so but it must be borne in mind that in none of the eighty-two West African natives—whether infected or uninfected—chosen at random, was the haemoglobin reading more than ninety, so that in any case, if the readings in this series were less than normal, this anaemia cannot be due to any of the three worms under consideration.

Conclusions regarding the influence of Ancylostome, Ascaris, and Trichuris infections on the haemoglobin percentage of eighty-four West African Natives.

1. A group of individuals with a low haemoglobin percentage does not show a greater percentage of infected cases than a group with a higher haemoglobin percentage.
2. A group of individuals with a low haemoglobin percentage does not show a greater average degree of infection than a group with a higher haemoglobin percentage.
3. Individuals with a high degree of infection do not necessarily show a low haemoglobin percentage.

This tolerance, so far as ankylostomiasis is concerned, would appear to be shared by some, at any rate, of the Indian races. Thus Mhaskar and Kendrick (1923), working in the tea estates of Madras, report as follows :—‘ There is no correlation between the haemoglobin average and the number of hookworms harboured; the presence of anaemia is not necessarily a sign of heavy infection.’ Chandler (1925), using Stoll’s technique, writes : ‘ In a study of 100 individuals in the Alipore Central Jail, Calcutta, sixty-seven of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gm. of faeces, no differences in haemoglobin percentage between the infected and uninfected individuals could be found.’

On the other hand, Stoll and Tseng (1925), working with Chinese cases, trace a definite connection between the number of ancylostomes harboured and the degree of anaemia; it is important to note, however, that the haemoglobin percentage of sixty-four ancylostome-free cases only averaged 66.7 per cent. and concerning these they write : ‘ The malaria is held to account for the low average haemoglobin of the hookworm negatives, and probably also influenced the degree of anaemia in the hookworm positives.’

(B) *Physique and General Fitness.* From a consideration of the figures in Table IV it is clear that the percentage of positive ancylostome cases is approximately equal in all three groups; on the other hand, there appears at first sight to be a very definite relationship between the average degree of infection and the physical standard of the group in which the cases occur; on consulting the column of maximum infections, however, it will be seen that the maximum infections occurring in Group B and Group C are much higher than that in Group A.

TABLE IV.

Showing the percentage of individuals infected with *Ancylostome*, *Ascaris*, and *Trichuris*, and the average degree of infection, in each of three groups of West African natives classified according to their physique and general fitness.

| Groups based on physique and general fitness | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of <i>Ancylostomes</i> per individual |
|--|--|--------------------------|---------|-----------|---|---------|-----------|---|---------|-----------|---|
| | | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | |
| A (Good) | For <i>Ancylostomes</i> only—32 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —50 | 84 | 22 | 44 | 2,619 | 2,418 | 102 | 15,600 | 42,630 | 933 | 109 |
| B (Moderate) | For <i>Ancylostomes</i> only—5 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —31 | 83 | 16 | 39 | 4,381 | 1,651 | 335 | 36,830 | 17,733 | 7,800 | 183 |
| C (Bad) | For <i>Ancylostomes</i> only—11 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —8 | 84 | 0 | 62 | 9,420 | 0 | 340 | 124,000 | 0 | 2,200 | 392 |

It is obvious that when one is dealing with a comparatively small number of cases a single pre-eminently heavy infection may be sufficient to raise to a considerable extent the average degree of infection of the whole group; and on enquiring into the question it was found that the maximum infections in Group B and Group C, shown in Table IV, were in fact outstanding, as the next highest infection in Group B was 23,100, and in Group C 17,760. Turning to a consideration of the *Ascaris* and *Trichuris* infections it was

similarly discovered that in each case there was a pre-eminently high infection, viz., 42,630 *Ascaris* ova per gm. in Group A, and 7,800 *Trichuris* ova in Group B. A truer picture is, therefore, obtained by omitting these predominantly high infections, and this is done in Table IVA.

TABLE IVA.

Table IV modified by the omission of the four predominantly high infections.

| Groups based on physique and general fitness | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of <i>Ancylostomes</i> per individual |
|--|---|--------------------------|----------------|------------------|---|----------------|------------------|---|----------------|------------------|---|
| | | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | |
| A (Good) | For <i>Ancylostomes</i> only—32 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —49 | 84 | 20 | 44 | 2,619 | 1,597 | 102 | 15,600 | 19,500 | 933 | 109 |
| B (Moderate) | For <i>Ancylostomes</i> only—5 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —30 | 83 | 16 | 37 | 3,453 | 1,651 | 86 | 23,100 | 17,733 | 1,000 | 144 |
| C (Bad) | For <i>Ancylostomes</i> only—10 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —8 | 83 | 0 | 62 | 3,054 | 0 | 340 | 17,760 | 0 | 2,200 | 128 |

It is seen from Table IVA that no definite correlation exists between the physical standard of a group and the percentage of infected cases, or the degree of infection occurring in that group.

Turning to the subject of the effect of intense infections, Table IV shows that the total number of cases examined for *ancylostomes* was 137; of these eighty-two (60 per cent.) fell into Group A, thirty-six (26 per cent.) fell into Group B, and nineteen (14 per cent.) into Group C. Eighty-nine natives were examined for *Ascaris* and *Trichuris* infections and were found to be distributed amongst the three groups in the following proportions; Group A, fifty (56 per cent.), Group B, thirty-one (35 per cent.), and Group C, eight (9 per cent.). It is now necessary to consider the distribution of the ten heaviest infections with each species of worm amongst the three groups, and to compare this distribution with that of the uninfected cases.

TABLE V.

Showing the ten heaviest *Ancylostome*, *Ascaris*, and *Trichuris* infections observed amongst West African natives, and the standard of physique and general fitness for each case.

| ANCYLOSTOME | | | | ASCARIS | | | TRICHURIS | | |
|-------------|----------------------|---------------------------------|--|---------|----------------------|---------------------------------|-----------|----------------------|---------------------------------|
| Case | Standard of physique | Number of ova per gm. of faeces | Computed number of <i>Ancylostomes</i> | Case | Standard of physique | Number of ova per gm. of faeces | Case | Standard of physique | Number of ova per gm. of faeces |
| 113 | C | 124,000 | 5,167 | 38 | A | 42,630 | 43 | B | 7,800 |
| 43 | B | 36,830 | 1,537 | 74 | A | 19,500 | 20 | C | 2,200 |
| 44 | B | 23,100 | 962 | 66 | B | 17,733 | 15 | B | 1,000 |
| 71 | B | 21,100 | 879 | 30 | A | 17,400 | 73 | A | 933 |
| 114 | C | 17,760 | 740 | 43 | B | 16,630 | 74 | A | 900 |
| 33 | A | 15,600 | 650 | 115 | B | 10,300 | 60 | A | 460 |
| 35 | B | 13,700 | 570 | 117 | A | 9,430 | 119 | A | 400 |
| 60 | A | 13,600 | 567 | 57 | A | 9,130 | 14 | A | 360 |
| 76 | A | 13,400 | 558 | 72 | B | 8,700 | 49 | B | 330 |
| 38 | A | 13,200 | 550 | 65 | B | 4,260 | 22 | A | 300 |

From Table V it can be seen that the ten heaviest infections with *Ancylostome*, *Ascaris*, or *Trichuris*, are in each case distributed according to the size of the group. Amongst the ten most intense *Ancylostome* infections 40 per cent. occur in Group A, 40 per cent. in Group B, and 20 per cent. in Group C; an examination of twenty-three natives who were not infected with *Ancylostomes* showed that 61 per cent. fell into Group A, 26 per cent. into Group B, and 13 per cent. into Group C. Of the ten heaviest *Ascaris* infections 50 per cent. occurred in Group A, and 50 per cent. in Group B; and of the seventy-three cases negative for *Ascaris*, 53 per cent. fell in Group A, 36 per cent. in Group B, and 11 per cent. in Group C. Amongst the ten heaviest *Trichuris* infections 60 per cent. occur in Group A, 30 per cent. in Group B, and 10 per cent. in Group C; fifty cases free from *Trichuris* infection occurred in the different groups in the following percentages, Group A, 56 per cent., Group B, 38 per cent., and Group C, 16 per cent. These figures, therefore, show no noticeable association between an intense infection with *Ancylostome*, *Ascaris*, or *Trichuris*, and a lowered standard of physique and general fitness.

Conclusions regarding the influence of Ancylostome infection on the physique and general fitness of 137 West African natives, and that of Ascaris, and Trichuris infections on eighty-nine of the same cases.

1. A group of individuals with a lower standard of physique and general fitness does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of physique and general fitness.

2. A group with a lower standard of physique and general fitness does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of physique and general fitness.

3. Individuals with a high degree of infection do not necessarily show a low standard of physique and general fitness.

(C) *Mentality.* It has already been stated in the Introduction that the mental examination was not directed to ascertaining how much the individual knew, but rather to discovering his mental alertness and ability to learn. In Table V are set out the percentage of infected cases, and the degree of infection, occurring amongst West African natives classified on this basis.

TABLE VI.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, amongst West African natives arranged in three groups according to their mentality.

| Groups based on mentality | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of Ancylostomes per individual |
|---------------------------|--|--------------------------|---------|-----------|---|---------|-----------|---|---------|-----------|--|
| | | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | |
| A (Good) | For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17 | 76 | 12 | 47 | 1,218 | 1,578 | 65 | 8,260 | 17,400 | 300 | 51 |
| B (Moderate) | For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—43 | 85 | 19 | 42 | 4,050 | 2,350 | 132 | 23,100 | 42,630 | 1,000 | 169 |
| C (Bad) | For Ancylostomes only—30 For Ancylostomes, Ascaris and Trichuris—29 | 85 | 21 | 45 | 5,193 | 1,523 | 392 | 124,000 | 17,733 | 7,800 | 216 |

The four predominant infections shown to be present in Table IV are, of course, also present in Table VI; the two Ancylostome infections and the one Trichuris infection occurring in Group C, and the predominant Ascaris infection in Group B. Omitting these four cases we have the results shown in Table VIA.

TABLE VIA.

Same as Table VI except that four predominantly high infections have been omitted.

| Groups based on mentality | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of Ancylostomes per individual |
|---------------------------|--|--------------------------|---------|-----------|---|---------|-----------|---|---------|-----------|--|
| | | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | Ancylostome | Ascaris | Trichuris | |
| A (Good) | For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17 | 76 | 12 | 47 | 1,218 | 1,578 | 65 | 8,260 | 17,400 | 300 | 51 |
| B (Moderate) | For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—42 | 85 | 17 | 42 | 4,050 | 1,391 | 132 | 23,100 | 19,500 | 1,000 | 169 |
| C (Bad) | For Ancylostomes only—29 For Ancylostomes, Ascaris and Trichuris—28 | 84 | 21 | 43 | 2,553 | 1,523 | 128 | 21,100 | 17,733 | 2,230 | 106 |

These figures of Ancylostome, Ascaris, and Trichuris infections in relation to mentality require careful consideration; in the first place it is clear that the percentages of Ancylostome and Trichuris infections are about equal in all three groups; the percentage of positive Ascaris cases is higher in Groups B and C, than in Group A, but the difference is not marked and the number of positive Ascaris cases dealt with is small. If we now consider the average degree of infection in the different groups, it is seen that in the case of Ascaris infection it is equal in all three groups, but that the Ancylostome and Trichuris infections are noticeably more intense in Groups B and C, than in Group A. In the Ancylostome infections Group B shows nearly four times, and Group C twice, as heavy an average degree of infection as Group A; this can hardly be explained on the assumption that Ancylostome infection has exerted a deleterious effect on the mentality, for if this were so we would expect to find that Group C was more intensely infected than Group B, whereas the

reverse is the case ; a similar state of affairs is also shown by the figures dealing with *Trichuris* infection. Table VI A, therefore, tends to disprove the theory that any relationship exists between the mentality of a group and the percentage of *Ancylostome*, *Ascaris* or *Trichuris* infected cases, or the degree of infection, occurring in that group.

It will be seen from Table VI that the proportionate sizes of the three groups in the mental classification bear no resemblance to those of the physical classification shown in Table IV ; in the mental classification the 137 natives examined are distributed amongst the three groups in the following proportions : Group A, twenty-five (18 per cent.), Group B, fifty-three (thirty-nine per cent.), Group C, fifty-nine (43 per cent.). Whereas all cases were examined for *Ancylostomes*, only eighty-nine cases were examined for *Ascaris* and *Trichuris* infections ; of these seventeen (19 per cent.) occurred in Group A, forty-three (48 per cent.) in Group B, and twenty-nine (33 per cent.) in Group C. Group A, therefore, forms much the smallest group in the mental classifications, whereas it formed much the largest group in the physical classifications. It is necessary to bear this fact in mind when considering the group distribution of the ten heaviest infections shown in Table VII.

TABLE VII.

Showing the ten heaviest *Ancylostome*, *Ascaris*, and *Trichuris* infections observed amongst West African natives, and the standard of mentality for each case.

| ANCYLOSTOME | | | | ASCARIS | | | TRICHURIS | | |
|-------------|-----------------------|---------------------------------|--|---------|-----------------------|---------------------------------|-----------|-----------------------|---------------------------------|
| Case | Standard of mentality | Number of ova per gm. of faeces | Computed number of <i>Ancylostomes</i> | Case | Standard of mentality | Number of ova per gm. of faeces | Case | Standard of mentality | Number of ova per gm. of faeces |
| 113 | C | 124,000 | 5,167 | 38 | B | 42,630 | 43 | C | 7,800 |
| 43 | C | 36,830 | 1,537 | 74 | B | 19,500 | 20 | C | 2,230 |
| 44 | B | 23,100 | 962 | 66 | C | 17,733 | 15 | B | 1,000 |
| 71 | C | 21,100 | 879 | 30 | A | 17,400 | 73 | B | 933 |
| 114 | C | 17,760 | 740 | 43 | B | 16,630 | 74 | B | 900 |
| 33 | B | 15,600 | 650 | 115 | A | 10,300 | 60 | B | 460 |
| 35 | B | 13,700 | 570 | 117 | B | 9,430 | 119 | B | 400 |
| 60 | B | 13,600 | 567 | 57 | C | 9,130 | 14 | B | 360 |
| 76 | B | 13,400 | 558 | 72 | B | 8,700 | 49 | B | 330 |
| 38 | B | 13,200 | 550 | 65 | C | 4,260 | 22 | A | 300 |

Table VII shows that the distribution of the ten heaviest Ancylostome infections is, Group B, 60 per cent., Group C, 40 per cent. ; of the twenty-three natives not infected with Ancylostomes, 26 per cent. fell into Group A, 35 per cent. into Group B, and 39 per cent. into Group C. The distribution of the ten heaviest Ascaris infections was 20 per cent. in Group A, 50 per cent. in Group B, and 30 per cent. in Group C ; seventy-three cases negative for Ascaris occurred in the three groups in the following proportions :—Group A, 21.5 per cent., Group B, 48.5 per cent., Group C, 30 per cent. Amongst the ten heaviest Trichuris infections, 10 per cent. occurred in Group A, 70 per cent. in Group B, and 20 per cent. in Group C ; of the fifty natives not infected with Trichuris, 18 per cent. fell into Group A, 50 per cent. into Group B, and 32 per cent. into Group C. Analysis of these figures shows that the ten heaviest infections are distributed according to the size of the groups ; they also show that the proportion of cases amongst the ten heaviest infections occurring in the C, or mentally bad group, corresponds very closely with the proportion of cases occurring amongst uninfected natives in the same group. Intense infection, therefore, with Ancylostome, Ascaris, or Trichuris, does not necessarily result in a lowered standard of mentality.

Conclusions regarding the influence of Ancylostome infection on the mentality of 137 West African natives and that of Ascaris and Trichuris infections on eighty-nine of the same cases.

The conclusions reached are the same as those for physique and general fitness.

Reference has already been made to Strong's (1916) interesting monograph on the effects of hookworm disease on the mental and physical development of school children. It is not clear where the experiments described were carried out, but presumably they were in one of the American States, and possibly dealt with a less resistant race than the West African native.

Butler (1915), working at the Bo School for the sons of Chiefs in Sierra Leone, wrote as follows : '*Examination for Ankylostomiasis.*—The same seventy-five boys were examined also for this condition, and in only one case was there a negative result ; that is, 98.6 per cent. showed the presence of ancylostome ova. I think these cases may be regarded as fairly heavy infections, because in fifty-nine of the cases (that is, roughly, 80 per cent.) the ova were found in a single

examination of crude faeces. None of the individuals showed any symptoms or signs suggestive of ankylostomiasis. Duffers and individuals of acute intelligence appeared equally infected, and the standard of the school sports is quite as high as the average English Public School, so that I could not detect any evidence suggesting that these individuals suffered any disability from harbouring the parasite at that particular time, though symptoms of ankylostomiasis might quite likely appear if the individual was placed under some untoward condition, such as semi-starvation, when the ancylostome toxins might get the upper hand.' Easmon (1923), writing of the same school, repeats Butler's observations. These two papers are not based on any exact data, and are not quoted here as supporting the theory regarding the non-pathogenicity of ankylostomiasis; they are referred to merely because the present writer believes that they represent the views of the majority of medical men in Sierra Leone.

(D) *Energy*. The definition of energy has already been given as the keenness with which an individual attempts any mental or physical task allotted to him. The data collected regarding the percentage and degree of infection amongst West African natives, classified in three groups on this basis, are set forth in Table VIII.

TABLE VIII.

Showing the percentage of individuals infected with *Ancylostome*, *Ascaris*, and *Trichuris*, and the average degree of infection, amongst West African natives arranged in three groups according to their energy.

| Groups based on energy | Number of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of <i>Ancylostomes</i> per individual |
|------------------------|---|--------------------------|----------------|------------------|---|----------------|------------------|---|----------------|------------------|---|
| | | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | |
| A (Good) | For <i>Ancylostomes</i> only—20 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —27 | 72 | 19 | 37 | 1,990 | 1,677 | 94 | 12,660 | 17,400 | 933 | 83 |
| (B) (Moderate) | For <i>Ancylostomes</i> only—0 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —33 | 85 | 18 | 45 | 4,064 | 2,586 | 99 | 15,600 | 42,630 | 900 | 169 |
| C (Bad) | For <i>Ancylostomes</i> only—28 | | | | | | | | | | |
| | For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —29 | 91 | 17 | 45 | 5,681 | 1,429 | 423 | 124,000 | 17,733 | 7,800 | 237 |

In Table VIII the predominant *Trichuris*, and the two predominant *Ancylostome* infections, both occur in Group C; while the predominant *Ascaris* infection occurs in Group B. If, as before, we omit these four infections, the results are as shown in Table VIIIA.

TABLE VIIIA.

Same as Table VIII except that four predominantly high infections have been omitted.

| Groups based on energy | Numbers of cases examined | Percentage infected with | | | Average number of ova per gm. of faeces | | | Maximum number of ova per gm. of faeces | | | Computed average number of <i>Ancylostomes</i> per individual |
|------------------------|--|--------------------------|----------------|------------------|---|----------------|------------------|---|----------------|------------------|---|
| | | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | <i>Ancylostome</i> | <i>Ascaris</i> | <i>Trichuris</i> | |
| A (Good) | For <i>Ancylostomes</i> only—20 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —27 | 72 | 19 | 37 | 1,990 | 1,677 | 94 | 12,660 | 17,400 | 933 | 83 |
| B (Moderate) | For <i>Ancylostomes</i> only—0 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —32 | 85 | 16 | 45 | 4,064 | 1,334 | 99 | 15,600 | 19,500 | 900 | 169 |
| C (Bad) | For <i>Ancylostomes</i> only—27 For <i>Ancylostomes</i> , <i>Ascaris</i> and <i>Trichuris</i> —28 | 91 | 17 | 43 | 2,963 | 1,429 | 160 | 23,100 | 17,733 | 2,230 | 123 |

Consideration of Table VIIIA shows that *Ascaris* and *Trichuris* infections are in no way associated with a lowered standard of energy. As regards *Ancylostome* infection, it is seen that the percentage of infected cases in each group increases with each reduction in the standard of energy; but this increase in the percentage of infected cases is only as 72-85-91 and is, therefore, obviously too small to allow of any conclusions. The average degree of infection is higher in both Group B and Group C than it is in Group A, but, as was also found in the mental classification, the degree of infection in Group B is higher than in Group C, which represents a lower standard of energy, the ratio of A-B-C being as 2-4-3. Before studying the results of intense infection on the energy of the individual, as shown in Table IX, it is necessary to consider the proportionate sizes of the different energy groups shown in Table VIII; from this table it can be seen that, of 137 natives

examined for ancylostomes, forty-seven (34 per cent.) occurred in Group A, thirty-three (24 per cent.) occurred in Group B, and fifty-seven (42 per cent.) in Group C. Eighty-nine natives were examined for *Ascaris* and *Trichuris* infection; of these twenty-seven (30 per cent.) fell into Group A, thirty-three (37 per cent.) into Group B, and twenty-nine (33 per cent.) into Group C.

TABLE IX.

Showing the ten heaviest *Ancylostome*, *Ascaris*, and *Trichuris* infections observed amongst West African natives, and the standard of energy for each case.

| ANCYLOSTOME | | | | ASCARIS | | | TRICHURIS | | |
|-------------|--------------------|---------------------------------|--|---------|--------------------|---------------------------------|-----------|--------------------|---------------------------------|
| Case | Standard of energy | Number of ova per gm. of faeces | Computed number of <i>Ancylostomes</i> | Case | Standard of energy | Number of ova per gm. of faeces | Case | Standard of energy | Number of ova per gm. of faeces |
| 113 | C | 124,000 | 5,167 | 38 | B | 42,630 | 43 | C | 7,800 |
| 43 | C | 36,830 | 1,537 | 74 | B | 19,500 | 20 | C | 2,230 |
| 44 | C | 23,100 | 962 | 66 | C | 17,730 | 15 | C | 1,000 |
| 71 | C | 21,100 | 879 | 30 | A | 17,400 | 73 | A | 933 |
| 114 | C | 17,760 | 740 | 43 | C | 16,630 | 74 | B | 900 |
| 33 | B | 15,600 | 650 | 115 | A | 10,300 | 60 | B | 460 |
| 35 | B | 13,700 | 570 | 117 | A | 9,430 | 119 | B | 400 |
| 60 | B | 13,600 | 567 | 57 | B | 9,130 | 14 | A | 360 |
| 78 | B | 13,400 | 558 | 72 | B | 8,700 | 49 | C | 330 |
| 38 | B | 13,200 | 550 | 65 | A | 4,260 | 22 | A | 300 |

The figures in Table IX show that amongst the ten most intensely infected ancylostome cases, 50 per cent. fall into Group B and 50 per cent. into Group C; twenty-three natives were negative for ancylostomes and these were distributed in the proportions of 56 per cent. in Group A, and 22 per cent. in both Group B and Group C. The distribution of the ten heaviest *Ascaris* infections was, 40 per cent. in Group A, 40 per cent. in Group B, and 20 per cent. in Group C; seventy-three cases were negative for *Ascaris* and were distributed as follows: Group A, 30 per cent., Group B, 37 per cent., Group C, 33 per cent. Amongst the ten heaviest *Trichuris* infections 30 per cent. occurred in Group A, 30 per cent. in Group B, and 40 per cent. in Group C; fifty cases were negative for *Trichuris*; of these 34 per

cent. occurred in Group A, 34 per cent. in Group B, and 32 per cent. in Group C.

An examination of the figures in Table VIII has already shown that the numbers of natives examined for *Ascaris* and *Trichuris* infections were about equally distributed in the three groups. It will be seen from the figures above recorded that the intensely infected *Ascaris* and *Trichuris* cases, and also the cases free from these infections, likewise distribute themselves in more or less equal groups; that is to say, they occur in the proportions that would be expected if these two infections had no influence on energy.

The *Ancylostome* figures are of interest as they appear to indicate some connection between intense infection with *ancylostomes* and a lowered standard of energy; thus none of the ten heaviest infections occur in Energy Group A, whereas of the twenty-three uninfected cases, 56 per cent. fall into this category; Group C contains not only 50 per cent. of the ten heaviest infections, but the five most intense of these ten infections all fall into this class, whereas it contains only 22 per cent. of the uninfected cases. The figures seem to suggest, therefore, that very intense *Ancylostome* infections, represented by more than 15,000 ova per gm. of faeces, may possibly have a deleterious effect on the energy of the individual so infected.

Conclusions regarding the influence of Ancylostome infection on the energy of 137 West African natives, and that of Ascaris and Trichuris on eighty-nine of the same cases.

1. A group of individuals with a lower standard of energy does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of energy.

2. A group with a lower standard of energy does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of energy.

3. (a) Individuals with a high degree of infection with *Ascaris* or *Trichuris* do not necessarily show a low standard of energy.

- (b) The figures, such as they are, suggest that there may be some correlation between *Ancylostome* infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases; but it is obvious that to justify any definite conclusion of this kind the work must be repeated with very much larger groups of cases.

(E) *Urine*. Eighty-two natives—in whom the degree of infection with *Ancylostome*, *Ascaris*, and *Trichuris*, was already known—were examined for the presence of albumin and (or) casts in the urine; twenty-seven (33 per cent.) of the cases were positive for albumin; none of the cases showed the presence of casts. No association between the presence of *Ancylostome*, *Ascaris*, or *Trichuris* ova in the faeces and albumin in the urine could be demonstrated; nor was a high degree of infection with any of these worms necessarily associated with the presence of albumin in the urine. The high percentage of albuminurias is probably due to the frequent occurrence of chronic gonorrhoea amongst certain classes of the native population.

VI. EFFECT OF MIXED INFECTIONS

Mixed infections were common amongst the natives examined and it is obviously impossible to set forth briefly the results of different worm combinations. Tables showing the effects of mixed infections with any two species of worms under consideration have been prepared by the writer, with the result that no association has been demonstrated between any such double infection and a lowered standard of haemoglobin percentage, physique, mentality, or energy. Only five of the eighty-nine natives examined for *Ancylostome*, *Ascaris*, and *Trichuris* revealed the presence of all three infections in the one individual, and the full data regarding these five cases is set forth in Table X.

TABLE X.

Showing the degree of infection and the corresponding classification according to haemoglobin percentage, physique, mentality, and energy, of five cases of mixed infection with *Ancylostome*, *Ascaris*, and *Trichuris*, occurring amongst West African natives; also the presence or absence of albumin in the urine of such cases.

| Case | Number of <i>Ancylostome</i> ova per gm. of faeces | Number of <i>Ascaris</i> ova per gm. of faeces | Number of <i>Trichuris</i> ova per gm. of faeces | Haemoglobin group | Physique group | Mentality group | Energy group | Albuminuria |
|------|---|---|---|----------------------|-------------------|--------------------|-----------------|-------------|
| 38 | 13,200 | 42,630 | 33 | A (80%) | A | B | B | Negative. |
| 43 | 36,830 | 16,330 | 7,800 | — | B | C | C | Positive. |
| 64 | 363 | 366 | 163 | A (85%) | A | C | C | Negative |
| 74 | 333 | 19,500 | 900 | B (75%) | A | B | B | Negative. |
| 75 | 3,300 | 2,630 | 100 | A (80%) | A | C | B | Positive. |

Table X shows that mixed infections with *Ancylostome*, *Ascaris*, and *Trichuris*, are not necessarily associated with albuminuria or a lowered standard of haemoglobin percentage, physique, mentality, or energy.

VII. SUMMARY OF CONCLUSIONS

A study of the effects of ankylostomiasis on the health of 137 West African natives, and those of *Ascaris* and *Trichuris* on eighty-nine of the same cases, has shown that these infections, or a combination of these infections, produce no noticeable effects on the haemoglobin percentage, the physique and general fitness, or the mentality of the cases examined, nor is their presence in any way associated with albumin or casts in the urine. *Ascaris*, and *Trichuris* infections do not appear to be associated with a low standard of energy, nor are the percentage of *Ancylostome* infected cases, or the average degree of infection, necessarily noticeably greater in a group of individuals with a lower standard of energy than in one with a higher standard. On the other hand, the figures suggest the possibility of some association between *Ancylostome* infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases ; but only a few such intense infections were observed, and it is obvious that in order to justify any such definite conclusion the work must be repeated with very much larger groups of cases.

It therefore follows from these conclusions that before treatment, and especially before the so-called 'Mass treatment,' of ankylostomiasis, is applied to any native race, careful investigation should be made whether ankylostomiasis has any definite pathogenic effect on that race, and if pathogenic effects are noted, with what degree of infection they are associated.

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EXPLANATION OF PLATE VII

- FIG. 1. Showing the physical condition of six boys selected at random from amongst the negative or lightly infected *Ancylostome* cases.
- FIG. 2. Showing the physical condition of six boys selected at random from amongst the heaviest *Ancylostome* infections.



| | | | | | | | |
|---------------------------------------|----------------------|-------|-------|--------|-----|-------|-----|
| Number of Ova per gm. of faeces | <i>Ancylostome</i> | 433 | 0 | 200 | 900 | 0 | 0 |
| | <i>Ascaris</i> ... | 8,200 | 9,400 | 17,730 | 0 | 2,733 | 0 |
| | <i>Tricburis</i> ... | 0 | 100 | 0 | 0 | 400 | 166 |

FIG. 1



| | | | | | | | |
|---------------------------------------|----------------------|--------|--------|--------|--------|--------|--------|
| Number of Ova per gm. of faeces | <i>Ancylostome</i> | 36,830 | 23,100 | 21,000 | 15,600 | 13,200 | 13,700 |
| | <i>Ascaris</i> ... | 16,330 | 0 | 0 | 0 | 42,630 | 0 |
| | <i>Tricburis</i> ... | 7,800 | 0 | 0 | 0 | 33 | 66 |

A NEW VARIETY OF *ANOPHELES MARSHALLI*, FROM SIERRA LEONE

BY

A. M. EVANS

(Received for publication 14 November, 1925)

PLATE VIII

During a recent survey of the *Anophelini* in and around Freetown, Professor Blacklock and the author collected several larvae which gave rise to an *Anopheles* apparently related to *A. marshalli* Theo., but differing from it and its allies in certain characters. Further, the larvae were markedly distinct from those of *A. marshalli*, possessing palmate hairs on the thorax as well as on the first two segments of the abdomen, and it seems probable that larval characters will be found to be a good means of separating the closely allied species of the *marshalli* group.

Anopheles marshalli var. *freetownensis* n.var. (Plate VIII).

A variety of *A. marshalli* having the mesonotum chiefly clothed with hairs and the tarsi entirely dark.

FEMALE.

Head: occiput with the usual anterior-median white upright-forked scales and black or dark brown ones behind. *Palpi* with three white bands, the proximal one narrow and the two distal ones very broad and equal in length, apex white. Distal bands separated by about half the length of one of them, and extending so as to occupy rather more than the distal third of the palp. *Thorax*: integument dark greyish-brown, a thick group of long and narrow, curved white scales in the middle in front, rest of mesonotum clothed with pale brown hairs, interspersed with a few rather long, very narrow, curved, pale scales, the scales rather more numerous at the sides. *Abdomen*: blackish-brown with fine dark brown hairs. *Legs*. Entirely dark except apices of femora and tibiae which are

obscurely pale. *Wings* (Pl. VIII) : costa with six dark areas, the fifth rather long. First vein with light and dark areas coinciding with those of costa on distal half, but the white areas more extensive basally ; second vein with two white spots on the stem, one at the bifurcation and two on the upper branch ; third vein with two dark areas, a large one sub-basally and a small one sub-apically ; fourth vein with a large and a small white area on the stem, and one at the bifurcation, both branches white at the apex, the upper with an additional small white spot ; fifth vein with the stem mostly white-scaled, one dark area towards the base and a large one at the bifurcation involving the base of the upper fork, which has two more dark areas, lower branch white on basal two-fifths and at apex ; sixth vein with two large white areas on basal half, distal half dark. Fringe at the apex of the wing largely white, but a small dark spot present just above the apex of the lower branch of the second vein. White spots at the apices of the branches of the fourth and fifth vein, but not at the apex of the sixth. Scales relatively rather short and narrow, the longer lateral squames near the apex of the third vein measuring 0.05 mm. in length, and having the greatest width about one-fifth to one-fourth of the length, and five or six striae. Wing length c. 3.5 mm.

MALE.

Palpi. Long segment with sub-median ring and apex white ; last two segments white-scaled with narrow, basal black rings. Other scale characters as in female except that the wing scales are shorter and less dense.

Type : ♂ and ♀, bred from larvae taken in a stream, Kissy Bridge, near Freetown, Sierra Leone, 2.VII.25, Professor D. B. Blacklock and A. M. Evans. Other specimens, thirteen ♂♂ and fourteen ♀♀ from this and other localities near Freetown. Types in the collection of the Liverpool School of Tropical Medicine.

Variation. The most marked variation was exhibited by a female specimen in which the wings were considerably darker than in the majority of specimens. In the first vein the basal white area was interrupted by a dark spot opposite that on the costa, and the upper branch of the second vein was entirely dark.

The larva will be described in a joint paper by Professor Blacklock and the author, which is shortly to be published in these ANNALS.

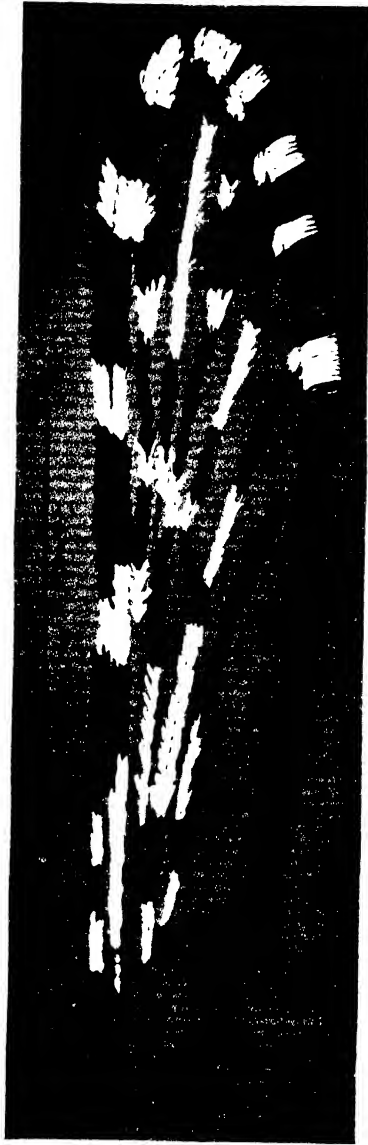
PLATE VIII

EXPLANATION OF PLATE VIII

Anopheles marshalli var. *freetownensis* n. var

Wing of female.

(The width of the white scales is slightly exaggerated.)



A.M.E., dei.

MISCELLANEA

ADDENDUM

With reference to my paper on 'A New Cestode from Nigeria,' in *Annals of Tropical Medicine & Parasitology*, Vol. XIX, No. 2, pp. 2 and 3, Dr. Joyeux has called attention to a species (*L. mahdiaensis*) described by him in *Archives de l'Institut Pasteur de Tunis*, Vol. XII, No. 2, p. 146.

Joyeux's species is distinct from those listed in my paper.

T. SOUTHWELL.

FILARIA MEDINENSIS

'The National Diseases here (Gold Coast of Guinea) are the *Small Pox* and *Worms*; . . . with the latter they are miserably afflicted in all parts of their Bodies, but chiefly in their Legs; which occasions a grievous Pain, which they are forced to bear till they can get the *Worm* quite out, that being sometimes a Month: The manner which the Artists take to get it out is this; as soon as the Worm is broken thro' the Tumour, his Head commonly first making its way, after they have drawn it out a little way, they make it fast to a stick, about which they every day wind a small part of it, till continuing this tedious Method, they have entirely wound out the whole, and the Patient is freed from his Pain. But if the Worm happens to break, they are put to a double Torture, the remainder part of the worm either rotting in the Body, or breaking out at some other place. The Negroes are most afflicted with these Worms: But though the Europeans are but seldom troubled with them, yet they do not escape them entirely. I have seen some Negroes who had nine or ten of them at once, with which

they were inexpressibly tormented. This *Worm-Disease* is frequent all the Coast over; but our Men are most tormented with it at *Cormantyn* and *Apam*; which perhaps may be occasioned by the foul Water which they are obliged to drink there. If you would know the length of these Worms, Monsieur *Focquenbrog* obligeth you with a pathetical Description; by which you are informed that they are some of them an Ell-long, and some as long as Pikes, and have not the patience to stay till the Man is dead, but seize him alive.'

(*A New and Accurate Description of the Coast of Guinea, divided into the Gold, the Slave, and the Ivory Coasts.* p. 108. Written originally in Dutch, by William Bosman, 1705. Reprinted for Sir Alfred Jones, K.C.M.G., at the Ballantyne Press, London, 1907.)

J. W. W. STEPHENS.

THE PREDACEOUS HABIT OF THE LARVAE *MUCIDUS SCATOPHAGOIDES*

The following interesting note on the predaceous habit of the larvae of this mosquito has just been received from Dr. Innes, of Bathurst.

'I am glad to be able to send you two ♂♂ and one ♀ *Mucidus scatophagoides*. I got them as large creamy-coloured larvae, in shallow grassy pools (rainwater) with some other culicine and anopheline larvae. Two pupated on the way to my office. Others pupated later. The pupa stage lasts about two days and the pupae also are creamy coloured. The larvae are larvivorous: I fed larvae to them which were all quickly devoured. I think this accounts for the very few larvae of other species of mosquitos which I found in the pools in association with these ogres.—FRANK A. INNES, Bathurst, Gambia, October 21st, 1925.'

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